

Evaluation of antioxidant enzymes activities during phytoremediation of textile waste water exposed of structurally different dyes by some aquatic plants

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To cite this article:

Al-Tae, M. M. S. and Witwit, R. T. A. Evaluation of antioxidant enzyme activities during phytoremediation of textile waste water exposed of structurally different dyes by some aquatic plants. *Mesop. Environ. J.*, 2015, Vol. 1, No. 2 , pp.35-65.

Abstract

The current study included investigate a response of five aquatic plants used to removed some of the pollutants found in an industrial waste water disposed from Textile Industry. The study conducted in laboratory for a period of 13 days and the species of aquatic plants were *Phragmites australis* , *Typha domingensis* , *Ceratophyllum demersum* , *Potamogeton Perfoliatus* and *Hydrilla varcillata* in polyethylene container and with addition two azo dye ,Reactive blue , Reactive yellow and mixture of theme in three concentrations of 0.01, 0.02 and 0.03 mg / L in each of theme .chlorophyll and protein content was estimated, Malondialdehyde (MDA) and activity of catalase (CAT) and superoxide dismutase (SOD) enzymes were estimated , the result show a decrease in total chlorophyll in species *Phragmites australis* , *Typha domingensis* , *Ceratophyllum demersum* , *Potamogeton Perfoliatus* and *Hydrilla varcillata* while decrease in protein content in species *Phragmites australis* , *Typha domingensis* , *Ceratophyllum demersum* , *Potamogeton Perfoliatus* and *Hydrilla varcillata* .the study showed high content (MDA) during the period of the experiment (0.67-4.04) , (0.64-5.98) , (9.56-31.6) , (12.709-61.29) and (0.88-34.53) nmole/mg *P. australis* , *T. domingensis* , *C. demersum* , *P. perfoliatus* and *H. varcillata* respectively. and increasing the activity of (CAT) during the period of the experiment ranged (43.9-140.7) , (8.8-101.4) , (25.5-136.7) , (23.3-129.7) and (33.4-85.5) unit/mg for plants *P. australis* , *T. domingensis* , *C. demersum* , *P. perfoliatus* and *H. varcillata* respectively. and increasing the activity of (SOD) enzyme during the period of the experiment ranged (0.086-0.53) , (0.23-3.46) , (0.608-8.46) , (3.89-15.2) and (0.411-4.35) unit/mg for plants *P. australis* , *T. domingensis* , *C. demersum* , *P. perfoliatus* and *H. varcillata* respectively. this response were due to environmental stress for instance contaminants, by developing a complex antioxidant defense system against abiotic stress by aquatic plants .

This study was conducted to determine the concentration of heavy metals(Fe, Cu, Zn, Ni, Cd, Cr and Pb) in canned tuna found in the local market of Hilla city, the results show that the concentrations of iron were above the limits and was ranged between 57.41 ppm to 101.55 ppm, while the concentration of copper was within the limits and was ranged between 5.43 ppm to 6.48 ppm, the concentration of zinc was also within the permitted limits and was ranged between 17.5 ppm to 37.5, while all of nickel, cadmium, chromium and lead was not detected in all the samples.

Keywords; phytoremediation , reactive blue and reactive yellow, aquatic plant , antioxidant enzymes , photosynthetic pigments.

Introduction

Synthetic dyes (e.g., azo dyes) are commonly used in the food, textile, cosmetic, plastic and pharmaceutical industries. These dyes designed to resist fading on exposure to soap, water, light and oxidizing agents, so that they can impart color on various raw materials. A majority of the synthetic dyes have been shown to disturb human health and are toxic to microorganism[1]. Hence, the treatment of effluents containing dyes has been a challenging problem among environmental technologies. Therefore, it is necessary to find an effective method to eliminate these dyes from industrial effluents. The physical and chemical methods have numerous disadvantages, such as high cost, low efficiency, and formation of toxic by-products. In the last decades, biological methods that use living organisms including bacteria, fungi, algae and plants were developed as a low-cost and ecofriendly viable alternative[2]. The use of plants to detoxify polluted environments (phytoremediation) referred to as phytotechnology, uses vegetation to contain, sequester, remove, or degrade inorganic and organic contaminants in soil, sediment, surface water, and groundwater.has attracted a lot of interest. Consequently, some plant species have been identified in possessing the high potential to degrade textile dyes [3].

The advantages of phytoremediation are the low capital costs, aesthetic benefits, minimization of leaching of contaminants, and soil stabilization . The operational cost of phytoremediation is also substantially less and involves mainly fertilization and watering for maintaining plant growth. In the case of heavy metals remediation, additional operational costs will also include harvesting, disposal of contaminated plant mass, and repeating the plant growth cycle.

textile industries about 10–15% of the dye gets lost in the effluent during the dyeing process[4] . Release of the colored effluents into the environment is undesirable as it affects the aesthetics,the water transparency and the gas solubility in water bodies[5] . More over amajority of these dyes are either toxic to flora and fauna or mutagenic and carcinogenic[6] .The effluents from the textile sector are characterized by high BOD, high COD, high concentrations of Total Dissolved Solids (TDS) and Total Suspended Solids (TSS),extreme pH (acidic or alkaline) and color which may distort the water quality,adds odor and significantly, hinders economic activities, making its proper treatment of a great concern . Textile industry generates highly polluting wastewater which contains dyes and their decomposition is creating very serious problem to wastewater treatment plant.

Dyes have complex aromatic structures to provide intense coloration, high water solubility, resistance to fading, improve delivery to the fabrics and to have variety of shades, which makes them highly resistant to degradation . phyto remediation is a green technology that uses plant systems for remediation and restoration of the contaminated sites. Plants have inbuilt enzymatic machinery capable of degrading complex structures and can be used for cleaning of the contaminated sites.It is an ecologically sound and sustainable reclamation strategy for bringing polluted sites into productive use but is still in experimental stage; therefore it needs a lot of attention and scientific scrutiny [7].

Materials and methods

Plant material and water sample preparation

five types of aquatic plants *P. australis* , *T. domingensis* , *C. demersum* , *P. perfoliatus* and *H. varcillata* collected from the drainage and washed with water drainage and well preserved in bags of clean

polyethylene while access to the lab [8] .

Fifteen plastic container size (15 liters) coated on the inside with aluminum foil were used and added (10) liters of textile industrial waste water were added in three concentrations (0.01, 0.02, 0.03)mg/L of the dyes Azo dyes (Reactive blue, Reactive yellow and Mixture from it) and with three replicates for each concentration will added and two control used the first containing industrial water only without plant and the second containing tap water with plant (250) g of aquatic plant used in the experiment Adapted in aqueous systems containing tap water for two weeks and then wash thoroughly with water and exposed to polluted water [9]. For a period of 13 days and testing during the days (1, 4, 7, 10 and 13) .

Plant material and water sample preparation

Biochemical assays

Estimation of antioxidants

CAT activity was determined by monitoring the decrease in absorbance at 240 nm due to dismutation of H₂O₂ followed the method used by [10].

SOD activity was determined by monitoring the decrease in absorbance at 420 nm followed the method used by [11] .

Lipid peroxidation determination

Lipid peroxidation was estimated by formation of malondialdehyde (MDA) and its reaction with thiobarbituric acid Used the method use by [12] .

Estimation of protein content

The protein content in the tissues of aquatic plants was estimated by using the method of Bradford using Bradford solution then absorbance was measured spectrophotometrically at 595 nm and expressed protein content mg/g tissue vegetarian [13] .

Photosynthetic pigments analysis

Total chlorophyll Was estimated in the tissues of aquatic plants according to the method used by [14] . and the soft tissue of plant crushed with 80% of acetone .The extracts were centrifuged for 10 min at 2000 ×g. totall Chlorophylls content were determined spectrophotometrically at 645 and 663 nm .

Results and discussion

Antioxidant enzymes responses

plants have developed a complex antioxidant defense system against abiotic stress. CAT is important antioxidative enzyme, present in the peroxisomes and mitochondria of cells, which degrades H₂O₂ to water and molecular oxygen, that catalyzed reaction by SOD. The CAT activity increase could be explained by plants adaptive mechanism to maintain H₂O₂ at a steady-state level within the cells [15]. The

present study showed high enzymatic activity for (CAT) during the period of the experiment ranged (43.9-140.7) , (8.8-101.4) , (25.5-136.7) , (23.3-129.7) and (33.4-85.5) unit/mg for plants *P. australis* , *T. domingensis* , *C. demersum* , *P. perfoliatus* and *H. varcillata* respectively. "Fig. 1-15".

Superoxide dismutase activity directly regulates the amount of ROS and appears as a first line of defense, converting superoxide radicals into H_2O_2 [16]. The present study showed an increase in activity might reflect an enhanced superoxide radical production under chemical stress condition the activity of enzyme (SOD) Superoxide dismutase in aquatic plants during the period of the experiment ranged (0.086-0.53) , (0.23-3.46) , (0.608-8.46) , (3.89-15.2) and (0.411-4.35) unit/mg for plants *P. australis* , *T. domingensis* , *C. demersum* , *P. perfoliatus* and *H. varcillata* respectively. "Fig. 16-30".

MDA is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage [17]. Thus, cell membrane stability has widely been utilized to study the effects of stress on plants [18]. The current study showed high content (MDA) Malondialdehyde in aquatic plants during the period of the experiment the change in MDA content were by (0.67-4.04) , (0.64-5.98) , (9.56-31.6) , (12.709-61.29) and (0.88-34.53) nmole/mg *P. australis* , *T. domingensis* , *C. demersum* , *P. perfoliatus* and *H. varcillata* respectively. "Fig. 31-45".

protein being an important organic constituent plays significant role in cellular metabolism and as constituents of cell membrane. It regulates the process of interaction between the intra and extracellular media [19]. It is known that soluble protein content is an important indicator of physiological status of plants[20] . Stressfull environments induce the generation of reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) etc . in plants there by creating a state of oxidative stress in them [21]. This increased ROS level in plants caused oxidative damage to biomolecules such as lipids, proteins and nucleic acids, thus, altering the redox homeostasis [22]. The protein content of the results show decrease in protein content during the period of the experiment (1.54-3.22) , (2.62-9.44) , (0.55-3.49) , (0.81-4.28) and (0.42-2.21) mg / g in weight for tender plants *P. australis* , *T. domingensis* , *C. demersum* , *P. perfoliatus* and *H. varcillata* respectively. "Fig. 46-60".

The total chlorophyll content of aquatic plants were decreased under dye stress condition may be a protective response to limit ROS by-product formation in chloroplasts [23]. during the period of the experiment from to (1.39-0.48) , (1.25-0.94) , (4.10-0.92) , (1.08-0.46) and (1.61-0.36) mg / g in weight for tender plants *P. australis* , *T. domingensis* , *C. demersum* , *P. perfoliatus* and *H. varcillata* respectively . "Fig. 61-75".

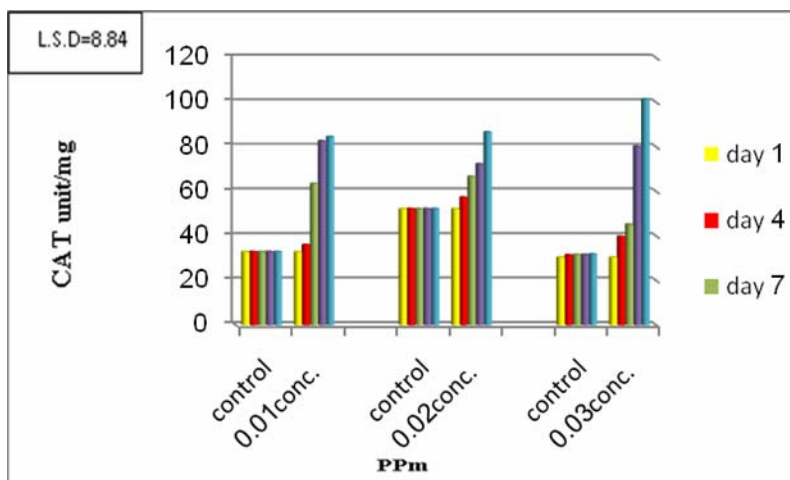


Figure 1. Variation in Catalase of *p.australis* that exposed in different concentration of Reactive Blue dye unit/mg

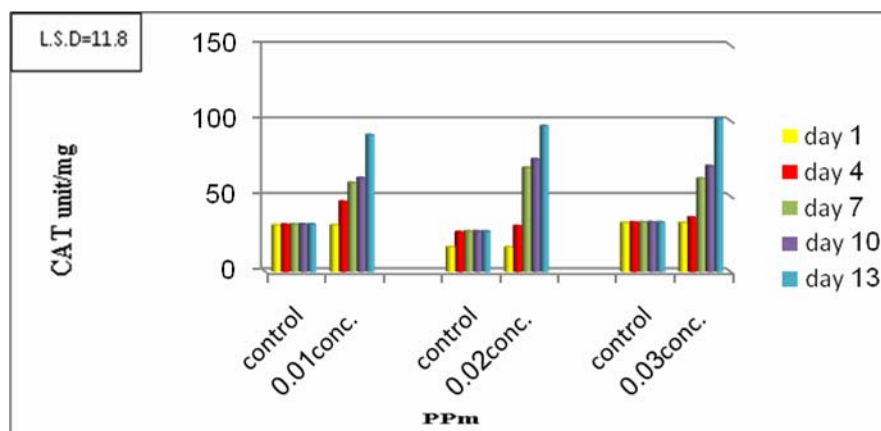


Figure 2. Variation in Catalase of *p.australis* that exposed in different concentration of Reactive yellow dye unit/mg

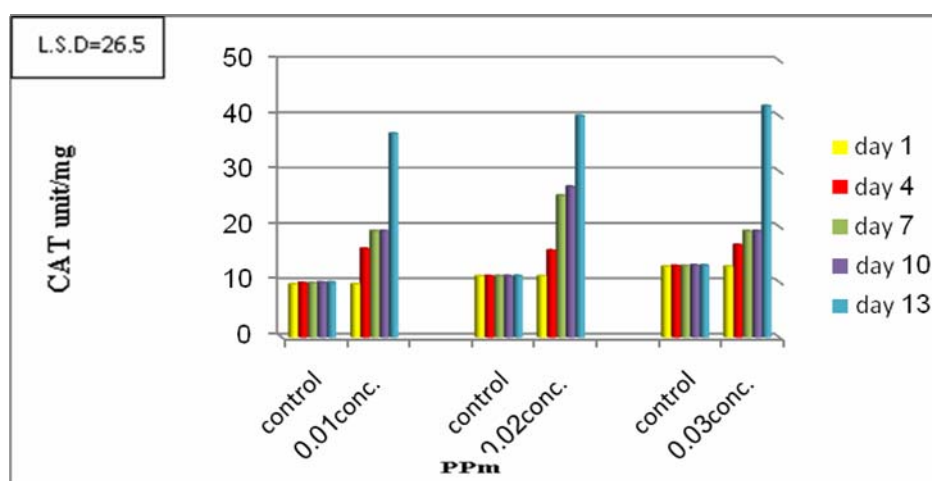


Figure 3. Variation in Catalase of *p.australis* that exposed in different concentration of mixture dye(Reactive Blue, Reactive yellow) unit/mg

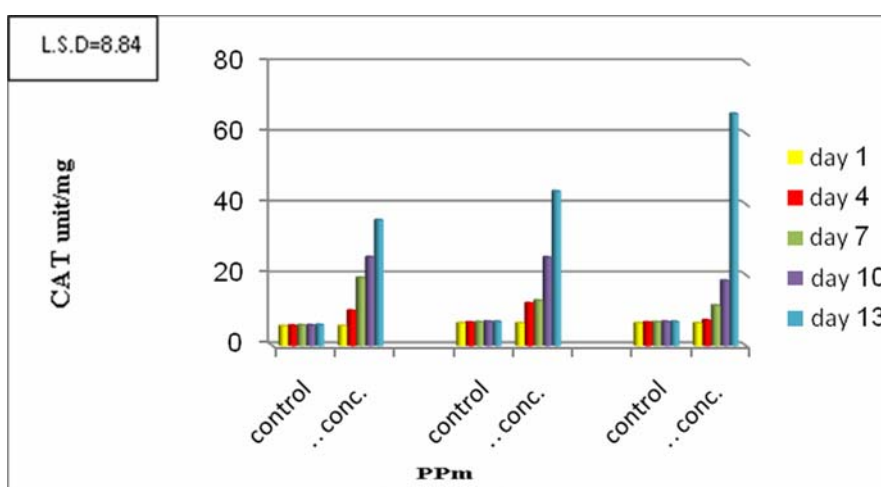


Figure 4. Variation in Catalase of *T.domingensis* that exposed in different concentration of Reactive Blue dye unit/mg

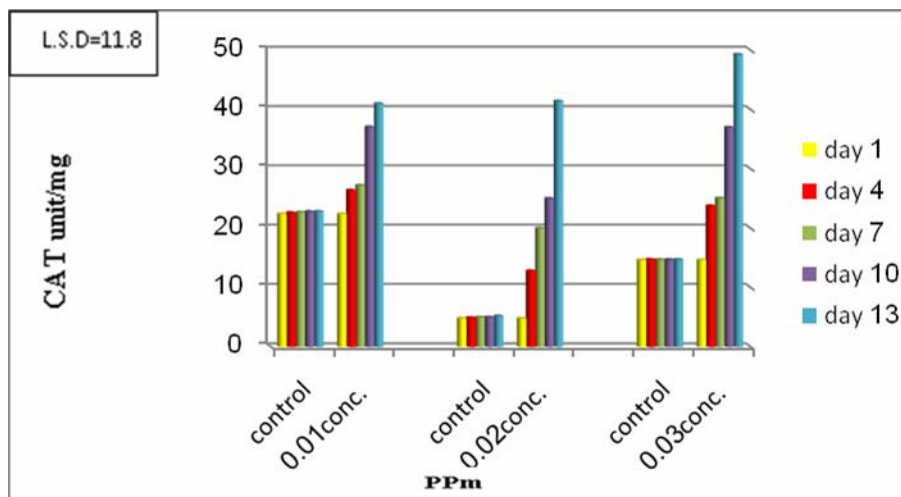


Figure 5. Variation in Catalase of *T.domingensis* that exposed in different concentration of Reactive yellow dye unit/mg

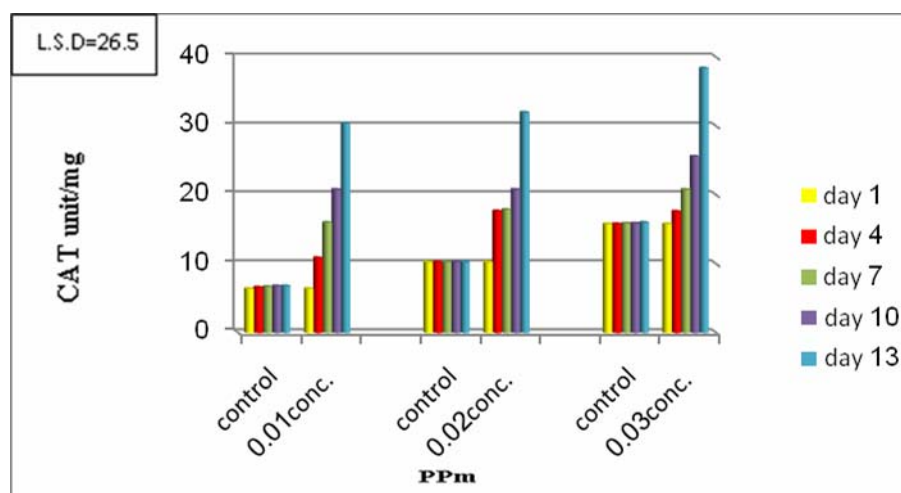


Figure 6. Variation in Catalase of *T.domingensis* that exposed in different concentration of mixture dye(Reactive Blue, Reactive yellow) unit/mg

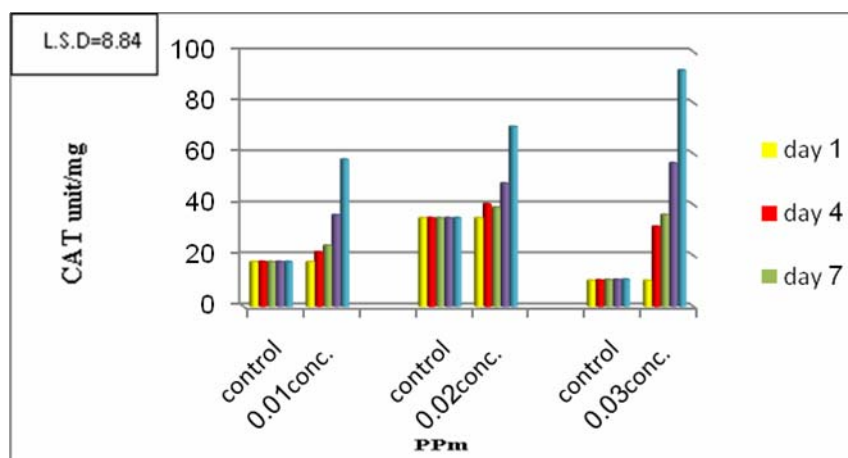


Figure 7. Variation in Catalase of *C. demersum* that exposed in different concentration of Reactive Blue dye unit/mg

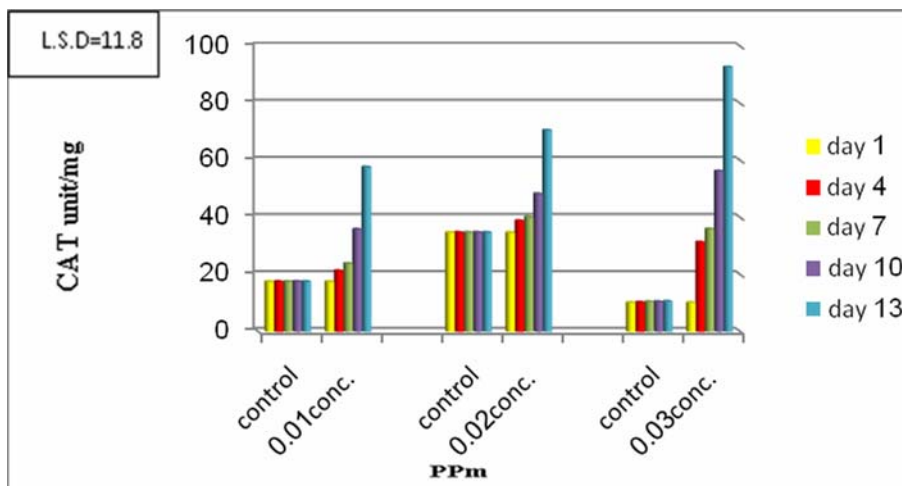


Figure 8. Variation in Catalase of *C. demersum* that exposed in different concentration of Reactive yellow dye unit/mg

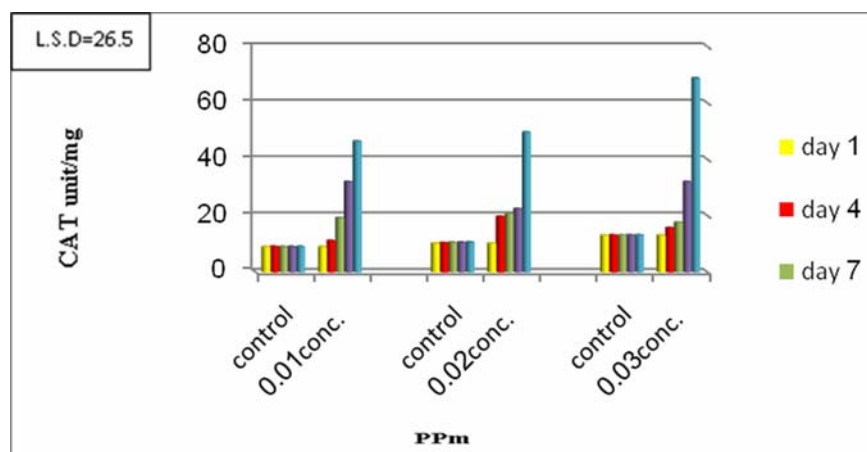


Figure 9. Variation in Catalase of *C. demersum* that exposed in different concentration of mixture dye(Reactive Blue, Reactive yellow) unit/mg

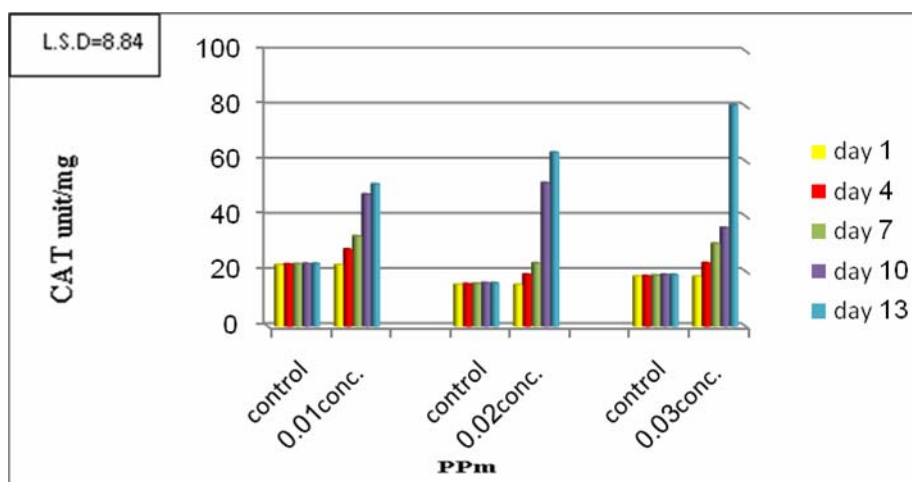


Figure 10. Variation in Catalase of *P. perfoliatus* that exposed in different concentration of Reactive Blue dye unit/mg

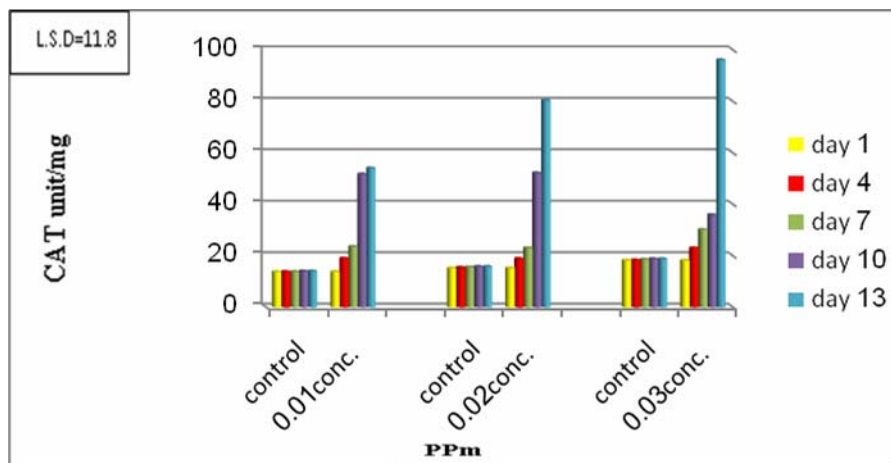


Figure 11. Variation in Catalase of *P. perfoliatus* that exposed in different concentration of Reactive yellow dye unit/mg

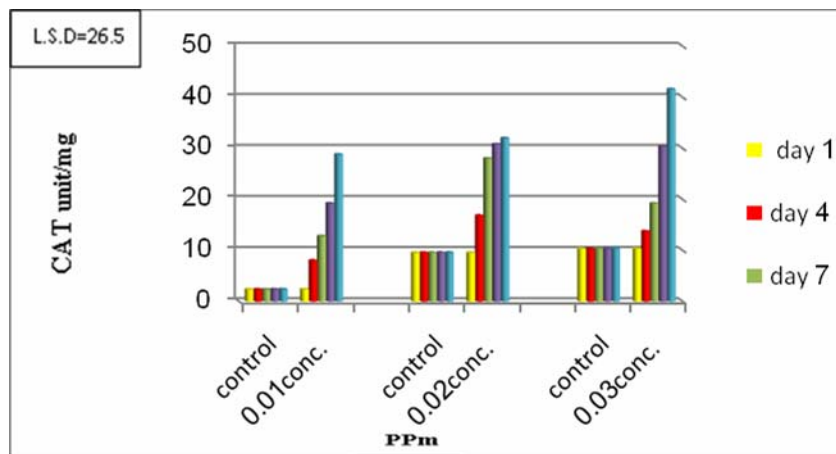


Figure 12. Variation in Catalase of *P. perfoliatus* that exposed in different concentration of mixture dye(Reactive Blue, Reactive yellow) unit/mg

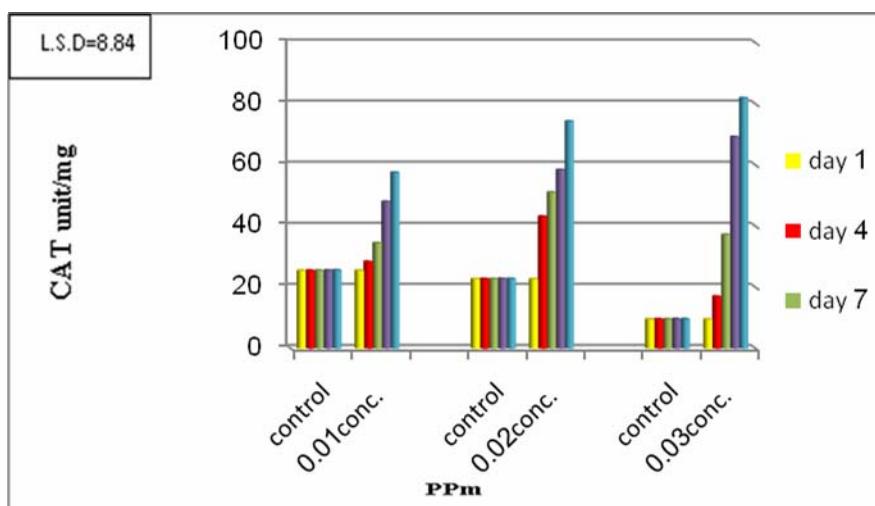


Figure 13. Variation in Catalase of *H. varcillata* that exposed in different concentration of Reactive Blue dye unit/mg

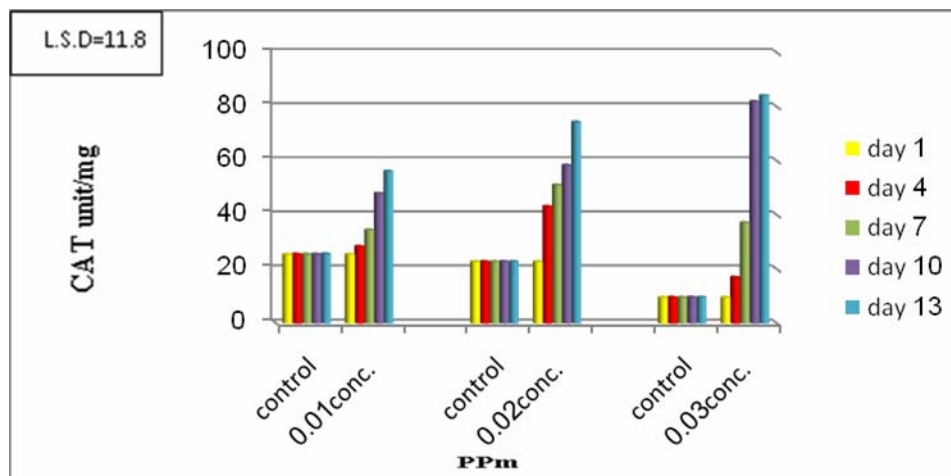


Figure 14. Variation in Catalase of *H. varicillata* that exposed in different concentration of dye Reactive yellow unit/mg

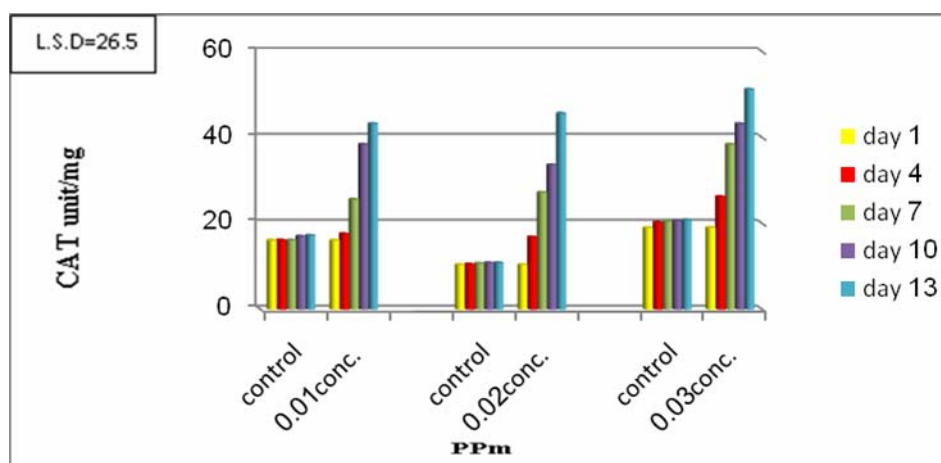


Figure 15. Variation in Catalase of *H. varicillata* that exposed in different concentration of mixture dye(Reactive Blue, Reactive yellow) unit/mg

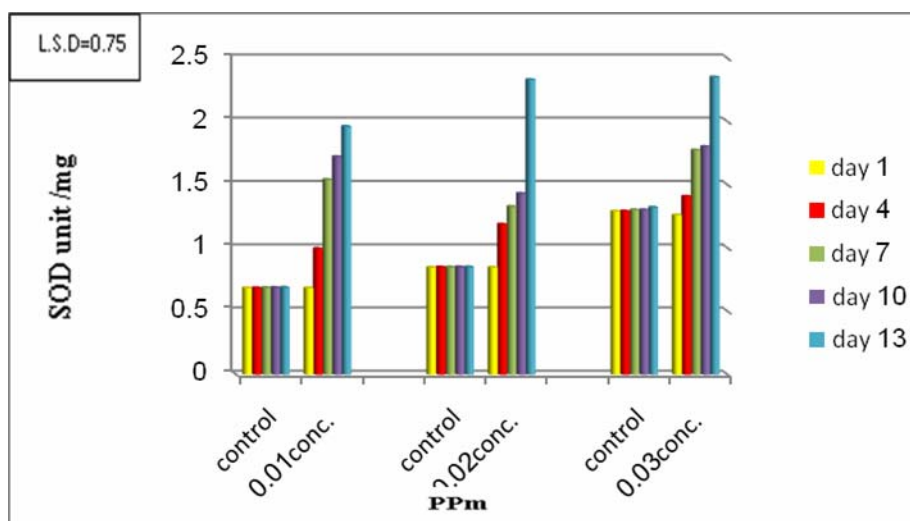


Figure 16. Variation in superoxide dismutase of *P. australis* that exposed in different concentration of Reactive Blue dye unit/mg

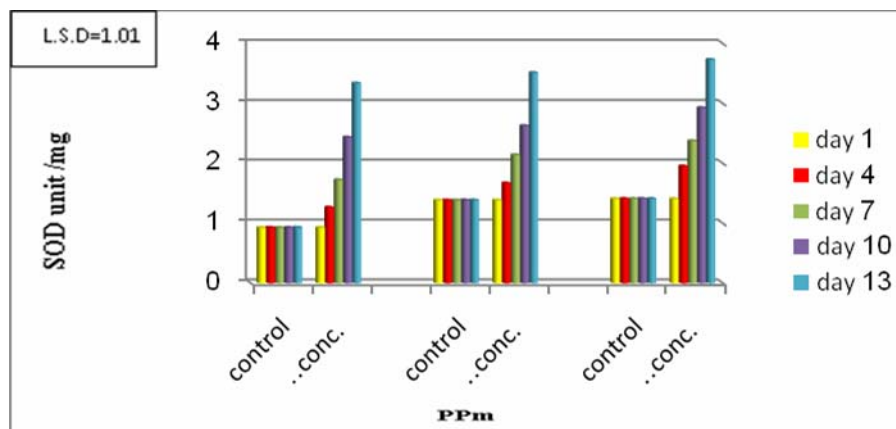


Figure 17. Variation in superoxide dismutase of *P. australis* that exposed in different concentration of Reactive yellow dye unit/mg

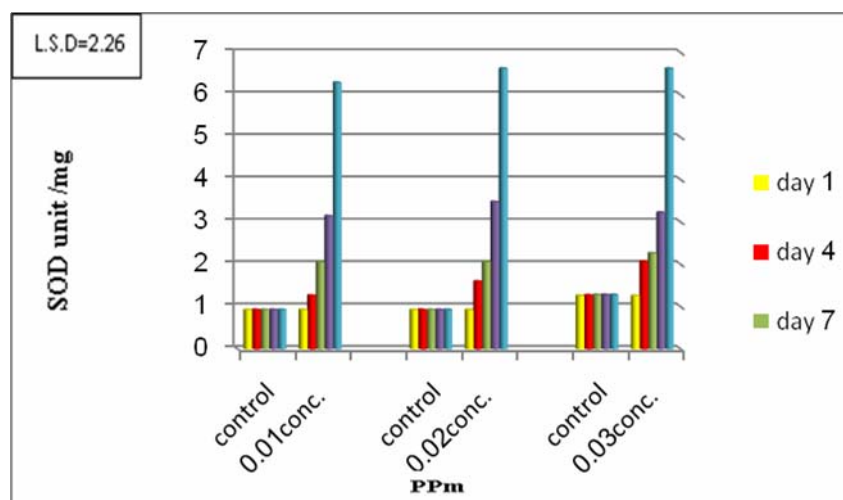


Figure 18. Variation in superoxide dismutase of *P. australis* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes unit/mg

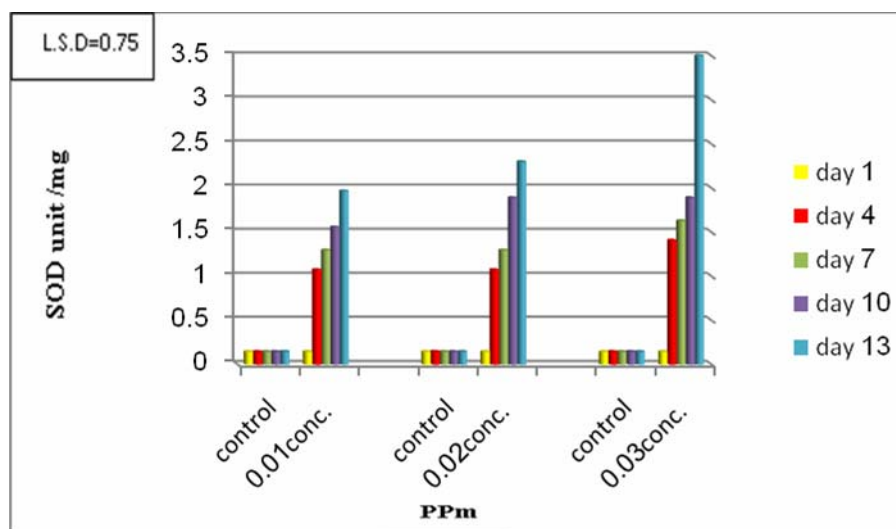


Figure19. Variation in superoxide dismutase of *T.domingensis* that exposed in different concentration of Reactive Blue dye unit/mg

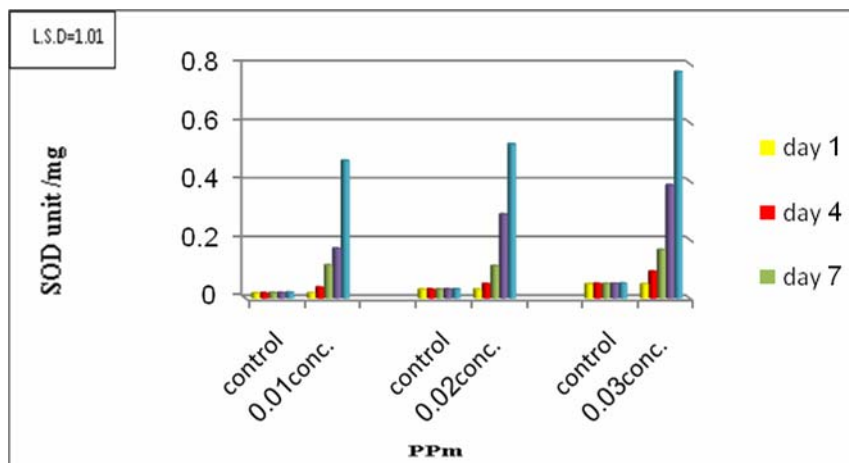


Figure20. Variation in superoxide dismutase of *T.domingensis* that exposed in different concentration of Reactive yellow dye unit/mg

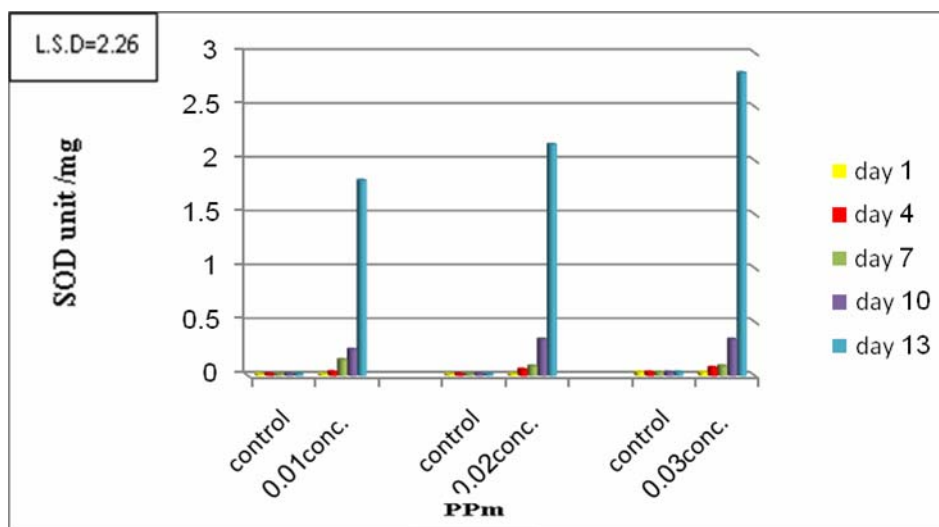


Figure 21. Variation in superoxide dismutase of *T.domingensis* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow dyes) unit/mg

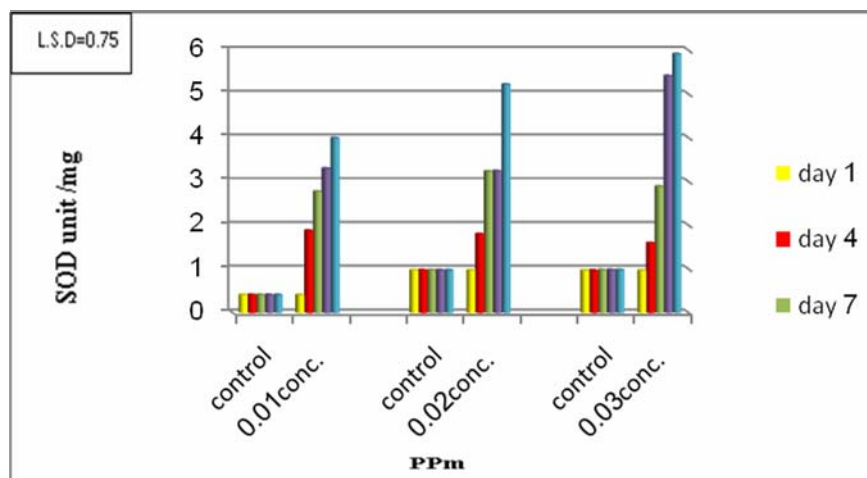


Figure 22. Variation in superoxide dismutase of *C. demersum* that exposed in different concentration of Reactive Blue dye unit/mg

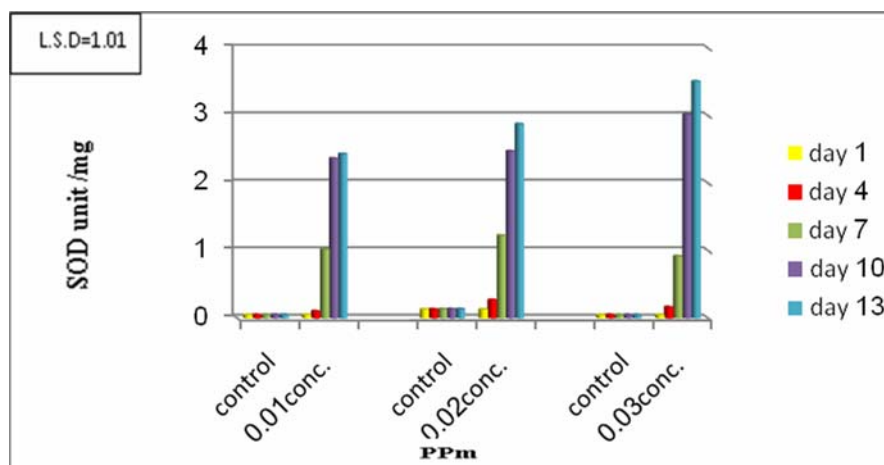


Figure 23. Variation in superoxide dismutase of *C. demersum* that exposed in different concentration of Reactive yellow dye unit/mg

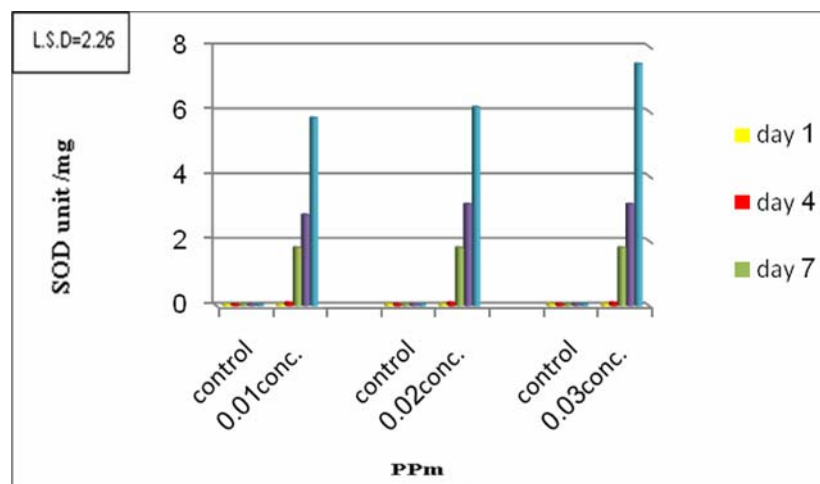


Figure 24. Variation in superoxide dismutase of *C. demersum* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes unit/mg

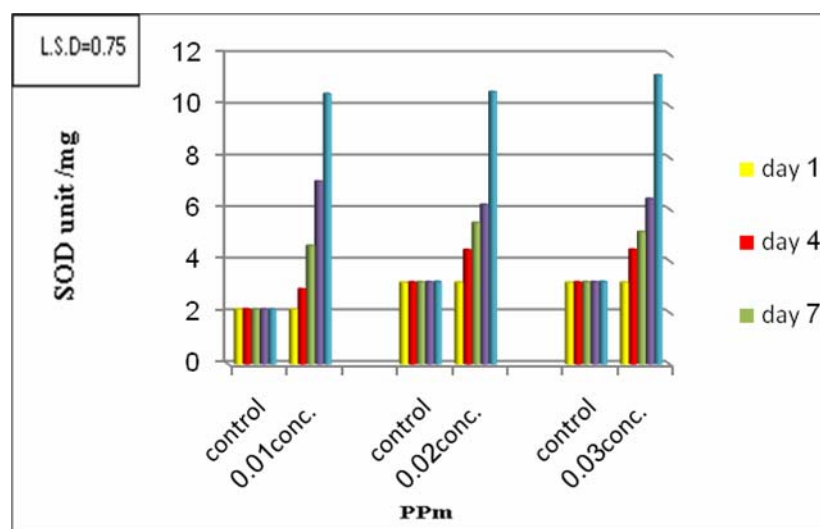


Figure 25. Variation in superoxide dismutase of *p. perfoliatus* that exposed in different concentration of dye Reactive Blue unit/mg

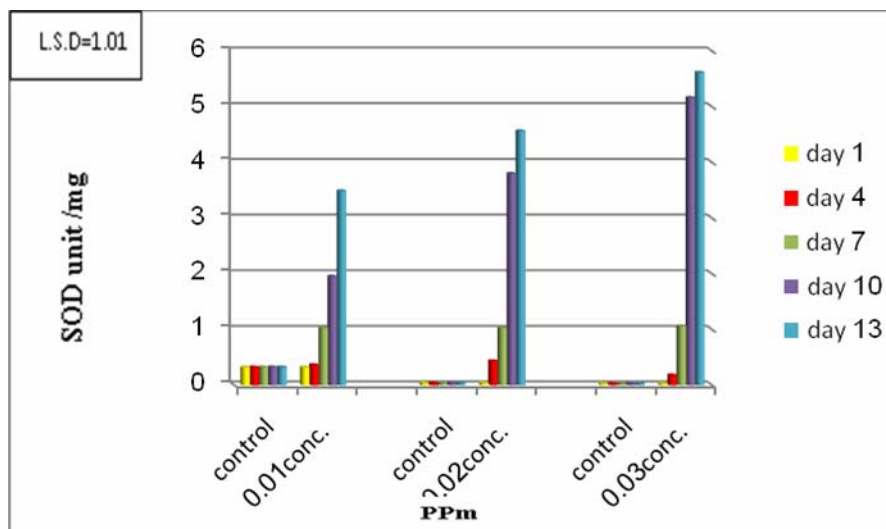


Figure 26. Variation in superoxide dismutase of *p. perfoliatus* that exposed in different concentration of Reactive yellow dye unit/mg

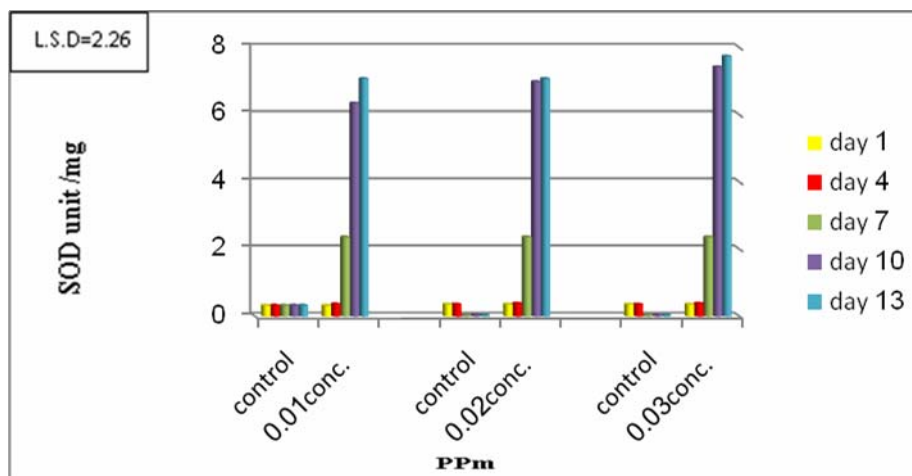


Figure 27. Variation in superoxide dismutase of *p. perfoliatus* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes unit/mg

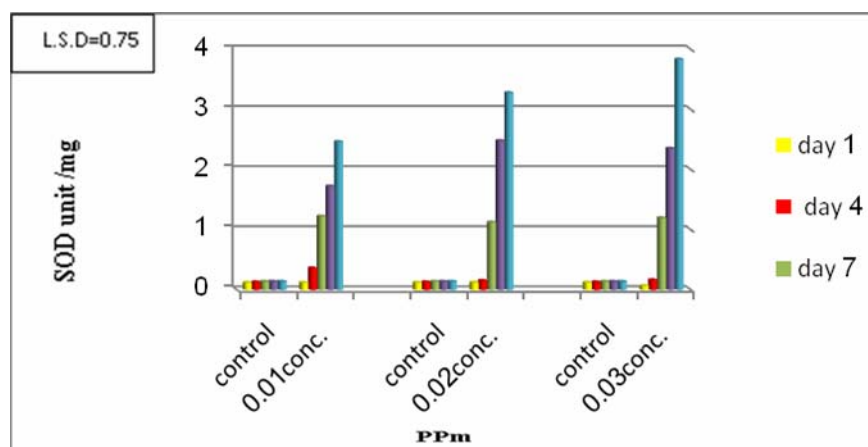


Figure 28. Variation in superoxide dismutase of *H. varcellata* that exposed in different concentration of Reactive Blue dye unit/mg

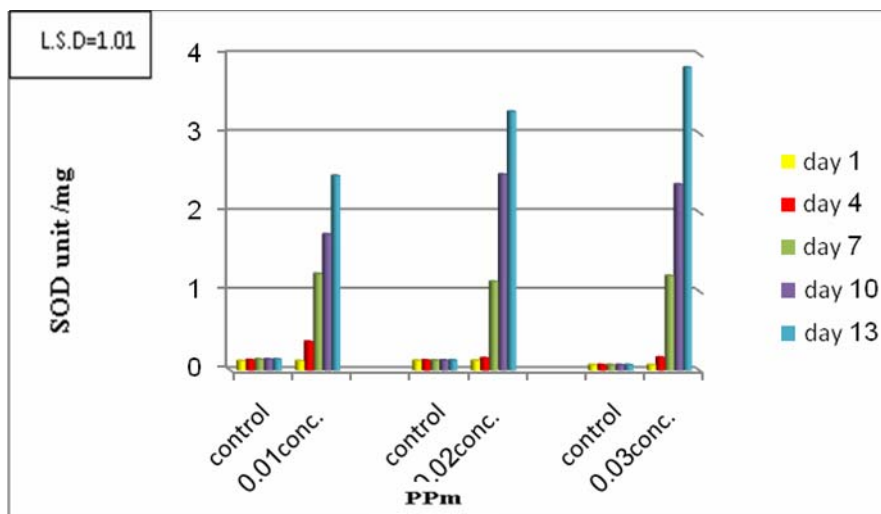


Figure 29 . Variation in superoxide dismutase of *H. varicillata* that exposed in different concentration of dye Reactive yellow unit/mg

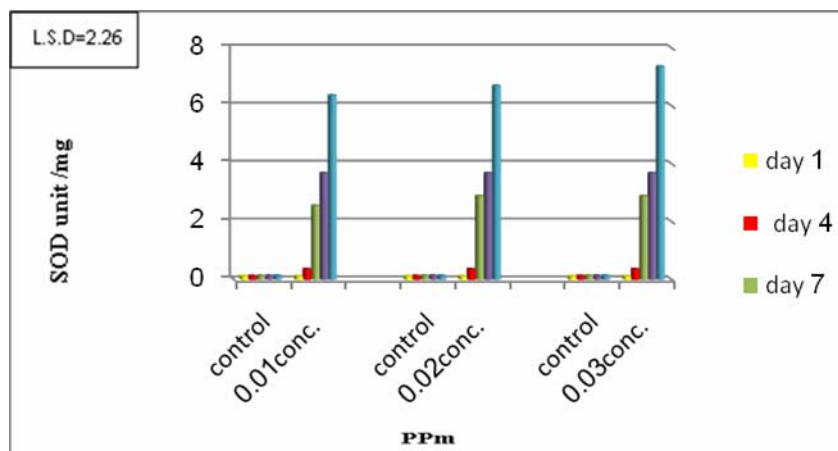


Figure 30 . Variation in superoxide dismutase of *H. varicillata* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes unit/mg

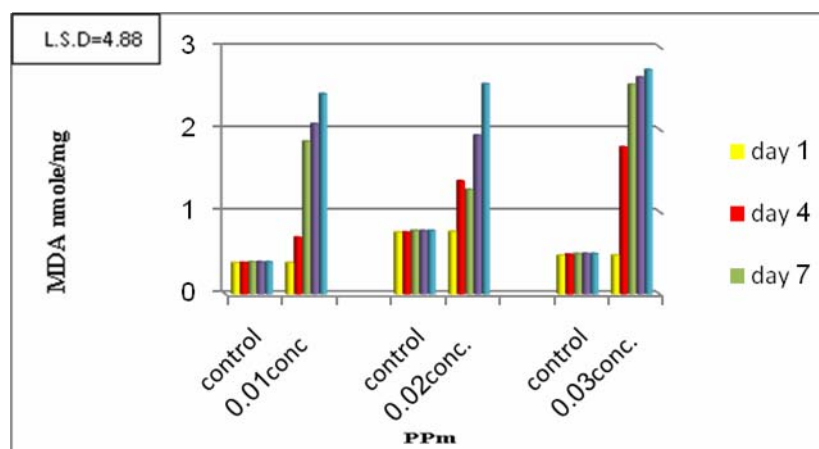


Figure 31. Variation in Malondialdehyde *P. australis* that exposed in different concentration of Reactive Blue dye nmole/mg

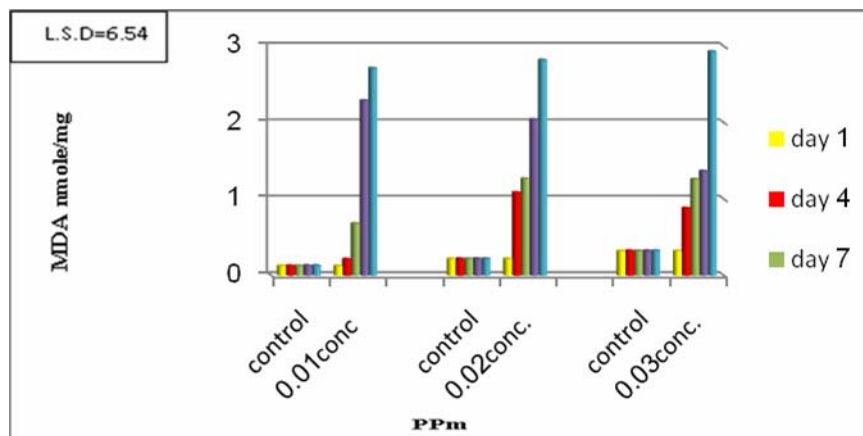


Figure 32. Variation in Malondialdehyde *P. australis* that exposed in different concentration of Reactive yellow dye nmole/mg

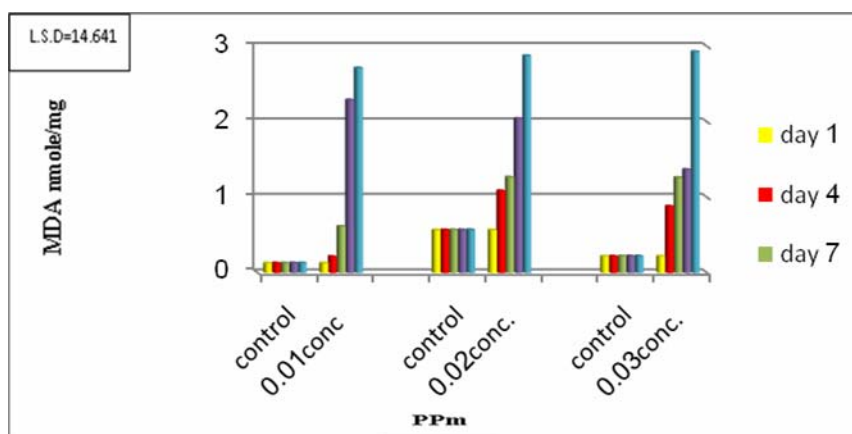


Figure 33. Variation in Malondialdehyde *P. australis* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes nmole/mg

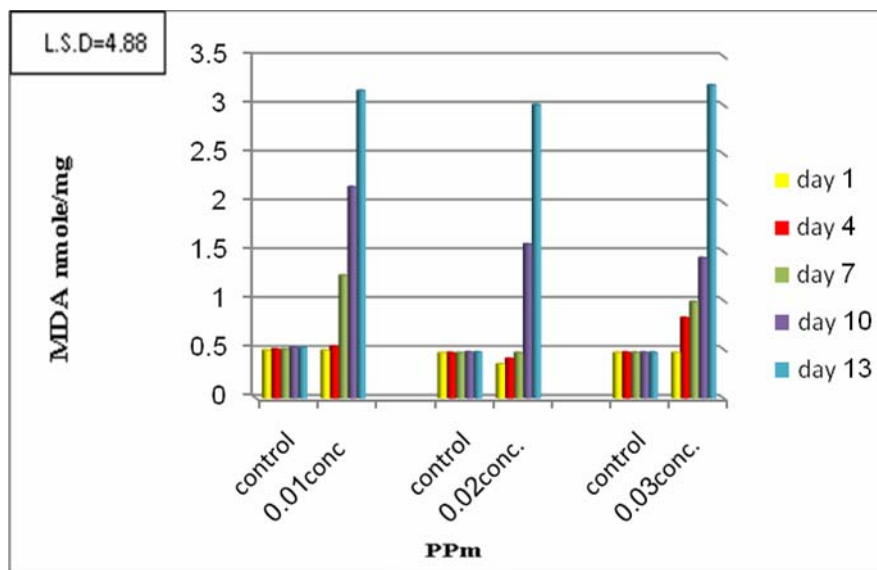


Figure 34. Variation in Malondialdehyde *T. domingensis* that exposed in different concentration of Reactive Blue dye nmole/mg

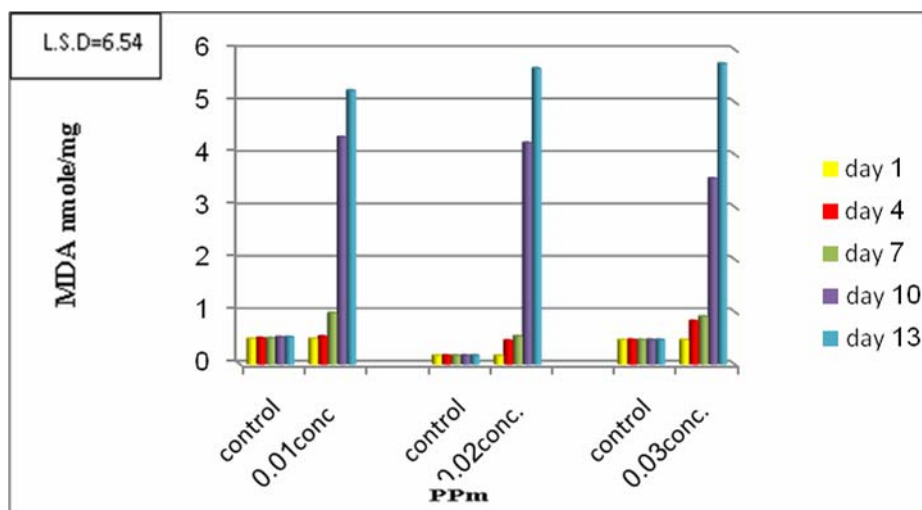


Figure 35. Variation in Malondialdehyde *T. domingensis* that exposed in different concentration of Reactive yellow dye nmole/mg

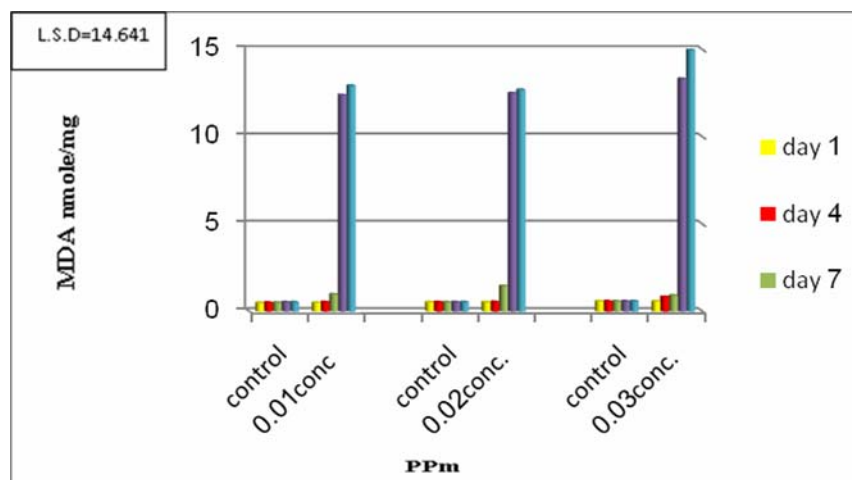


Figure 36. Variation in Malondialdehyde *T. domingensis* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes nmole/mg

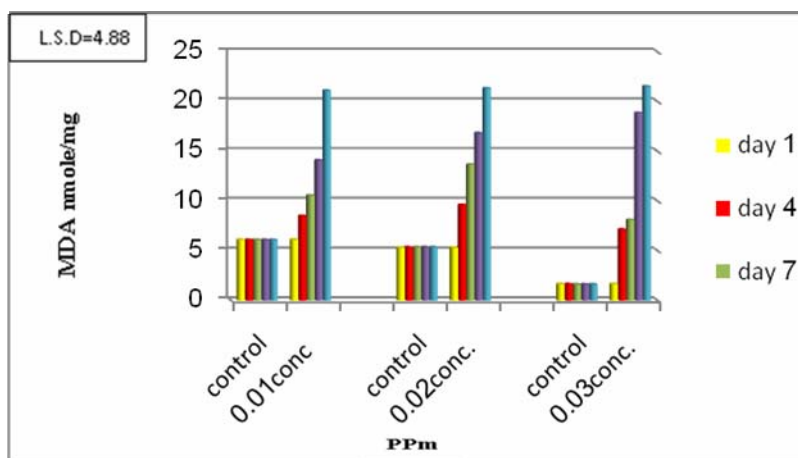


Figure 37. Variation in Malondialdehyde *C. demersum* that exposed in different concentration of Reactive Blue dye nmole/mg

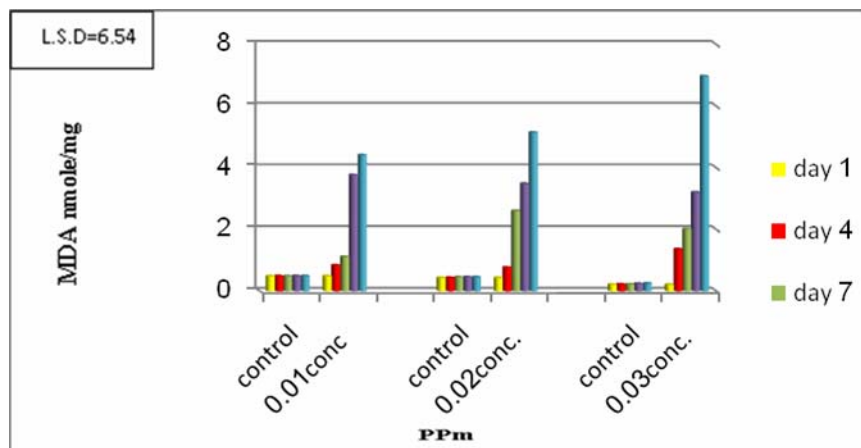


Figure 38. Variation in Malondialdehyde *C. demersum* that exposed in different concentration of Reactive yellow dye nmole/mg

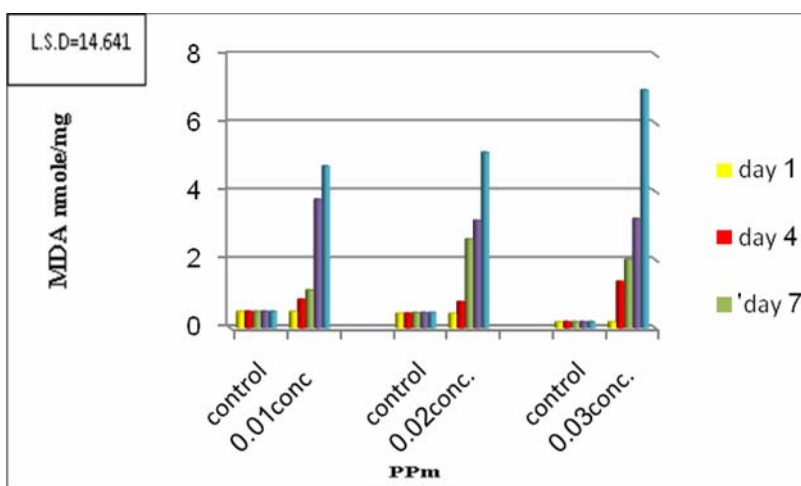


Figure 39. Variation in Malondialdehyde *C. demersum* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes nmole/mg

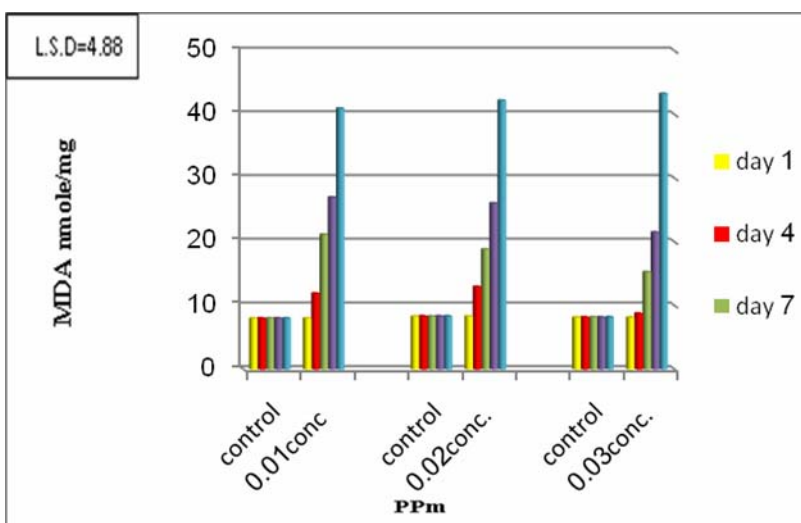


Figure 40. Variation in Malondialdehyde *P. perfoliatus* that exposed in different concentration of Reactive Blue dye nmole/mg

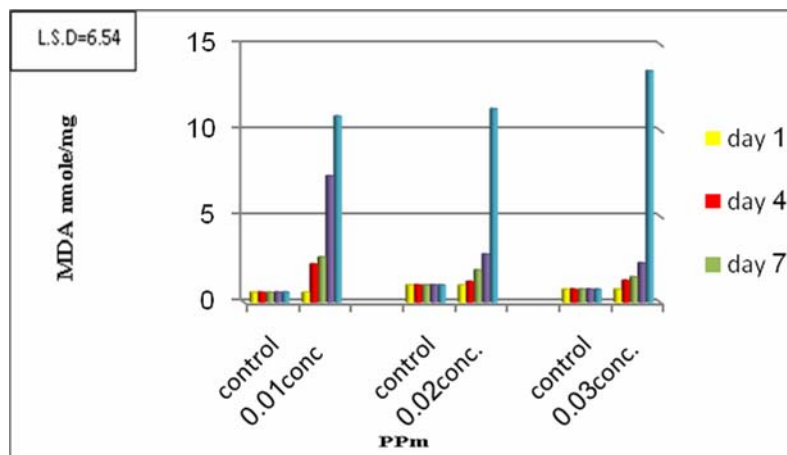


Figure 41. Variation in Malondialdehyde *P. perfoliatus* that exposed in different concentration of Reactive yellow dye nmole/mg

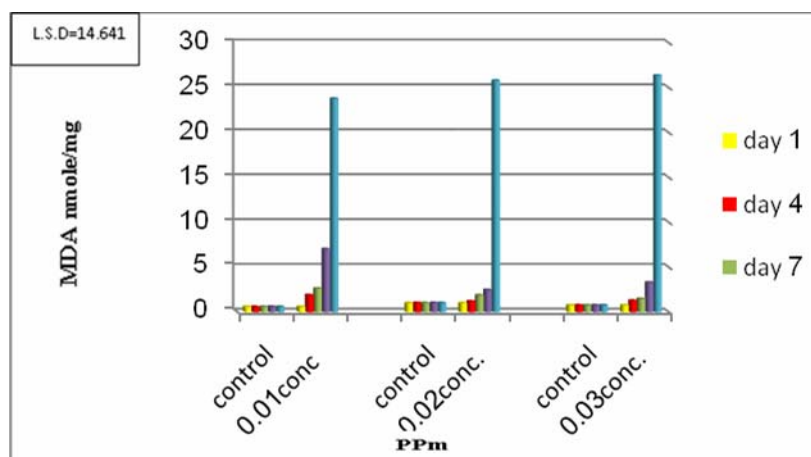


Figure 42. Variation in Malondialdehyde *P. perfoliatus* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes nmole/mg

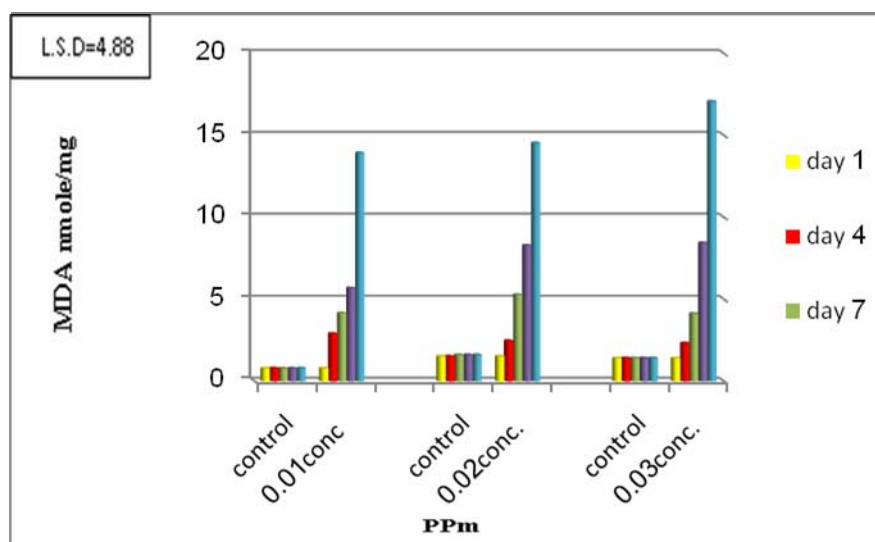


Figure 43. Variation in Malondialdehyde *H. varcillata* that exposed in different concentration of Reactive Blue dye nmole/mg

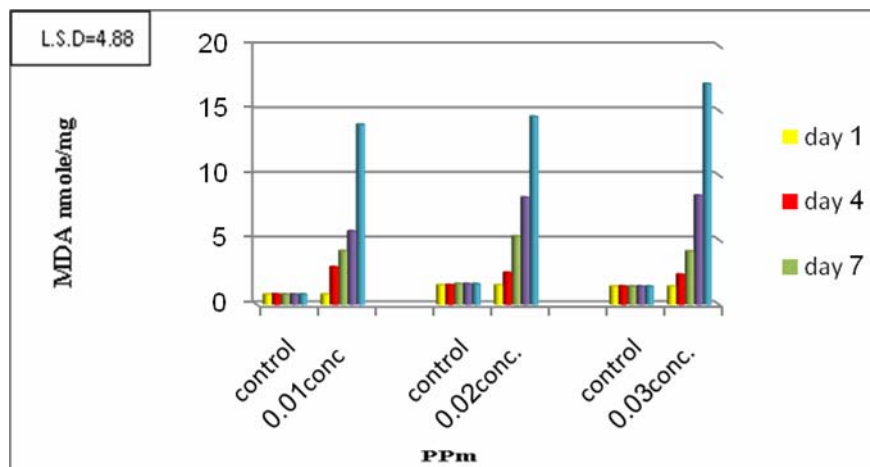


Figure 44. Variation in Malondialdehyde *H. varicillata* that exposed in different concentration of Reactive yellow dye nmole/mg

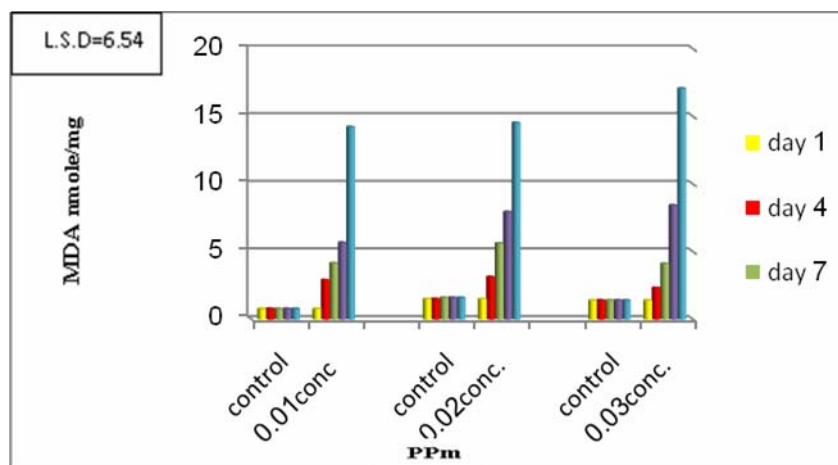


Figure 45. Variation in Malondialdehyde *H. varicillata* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes nmole/mg

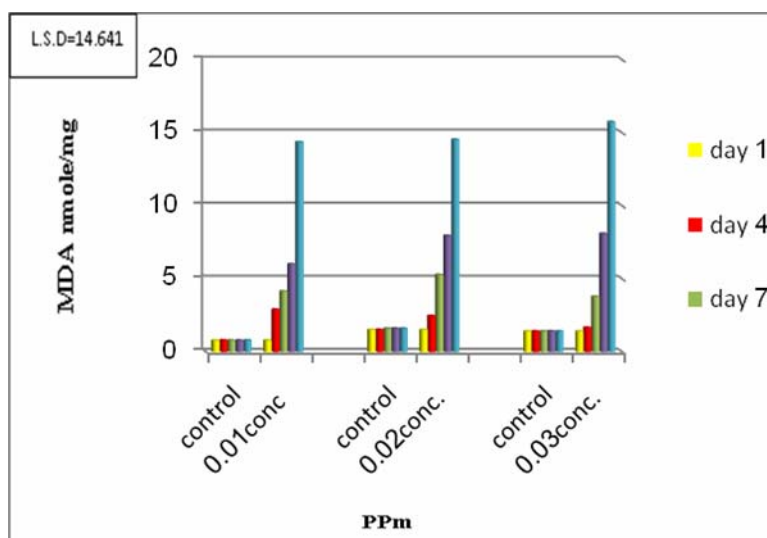


Figure 46. Variation in protein of *p.austeralis* that exposed in different concentration of Reactive Blue dye mg/gm

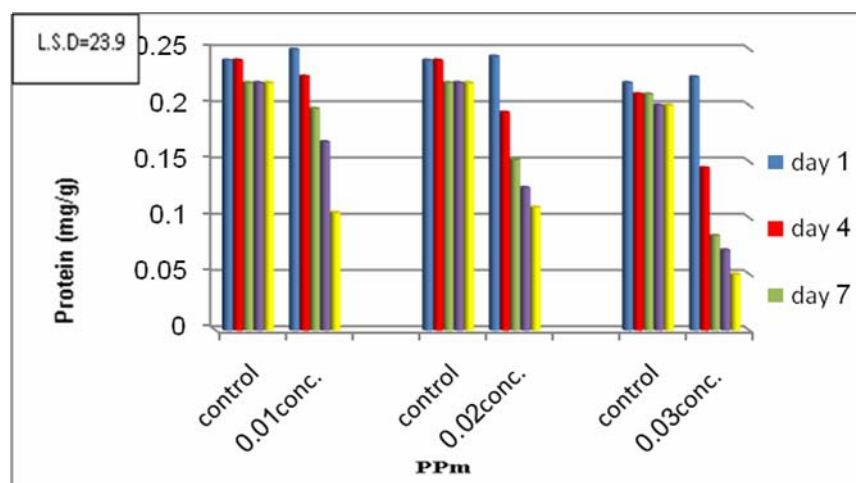


Figure 47. Variation in protein of *p.australis* that exposed in different concentration of Reactive yellow dye mg/gm

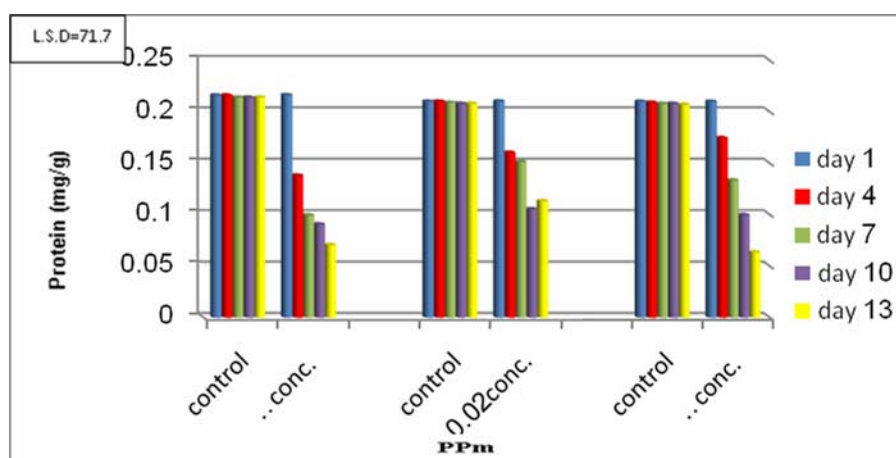


Figure 48. Variation in protein of *p.australis* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes mg/gm

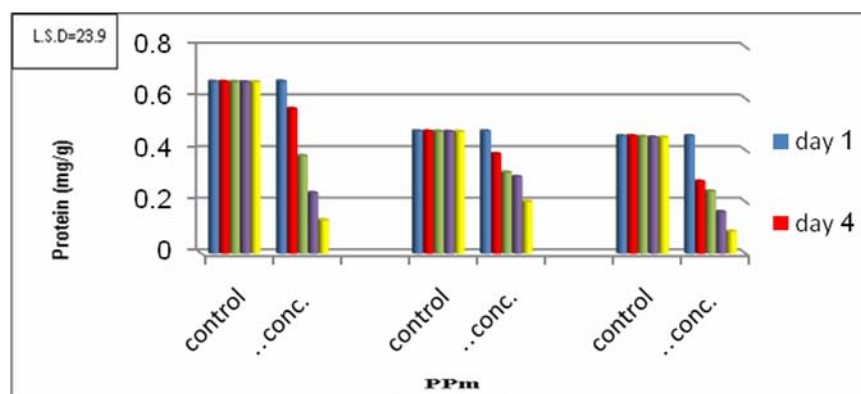


Figure 49. Variation in protein content of *T.domingensis* that exposed in different concentration of Reactive Blue dye mg/gm.

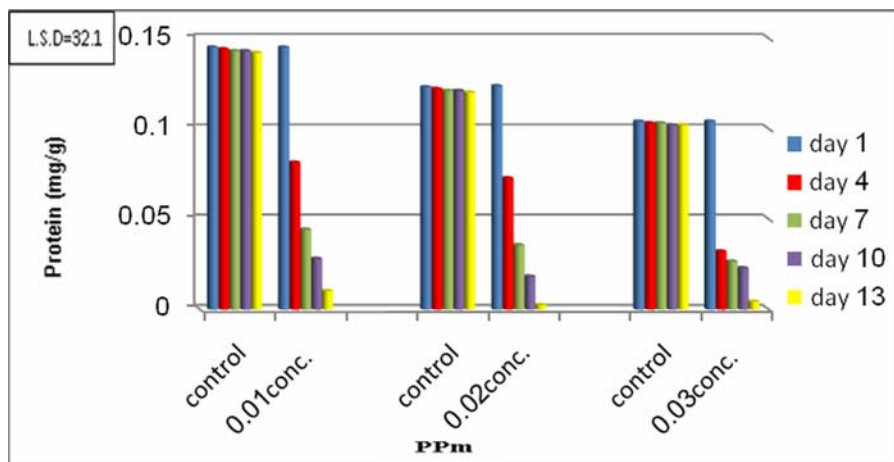


Figure 50. Variation in protein content of *T.domingensis* that exposed in different concentration of Reactive yellow dye mg/gm.

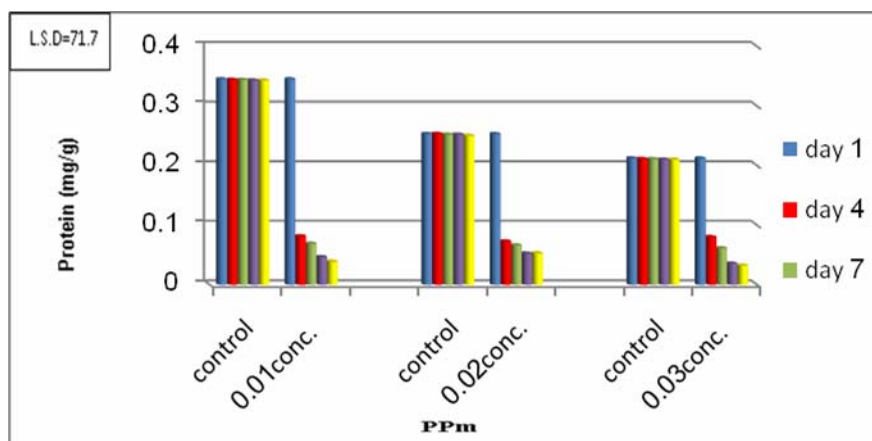


Figure 51. Variation in protein content of *T.domingensis* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes mg/gm.

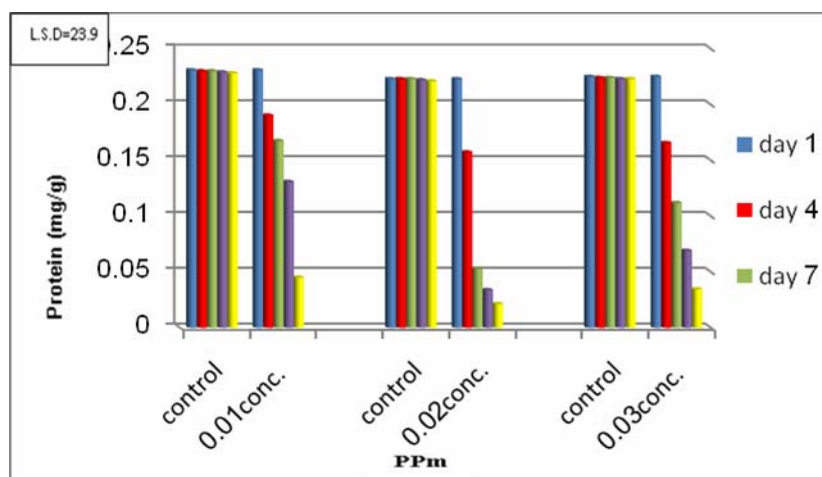


Figure 52. Variation in protein content of *C.demersum* that exposed in different concentration of Reactive Blue dye mg/gm

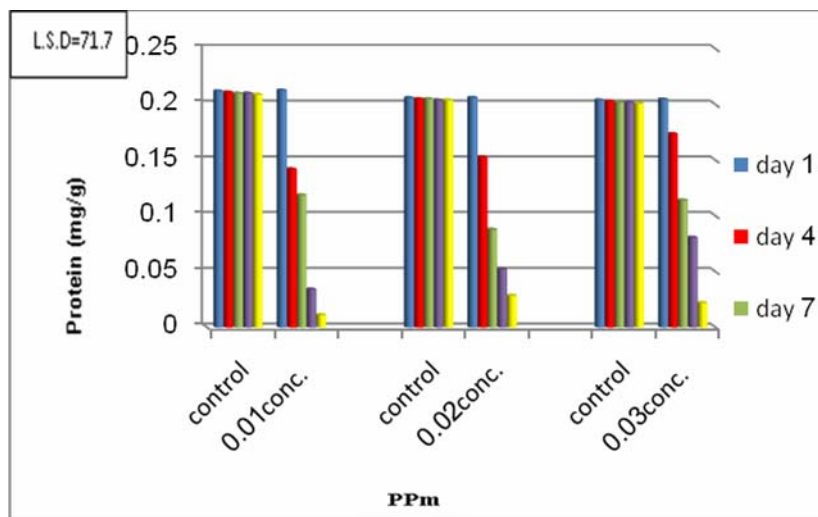


Figure 53. Variation in protein content of *C. demersum* that exposed in different concentration of Reactive yellow dye mg/gm

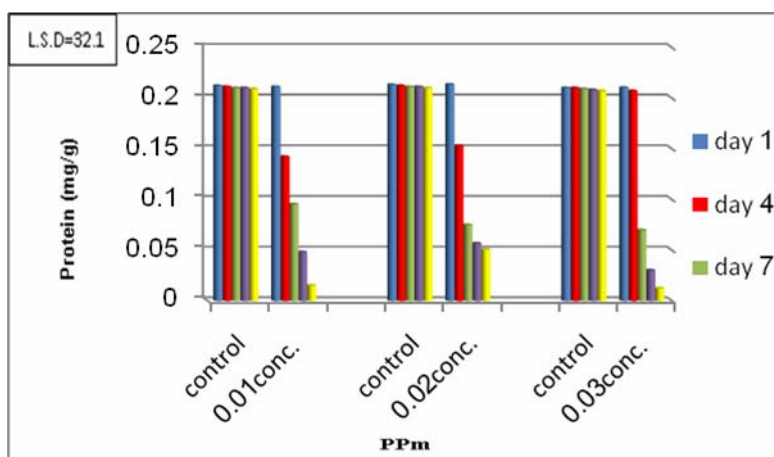


Figure 54. Variation in protein content of *C. demersum* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes mg/gm

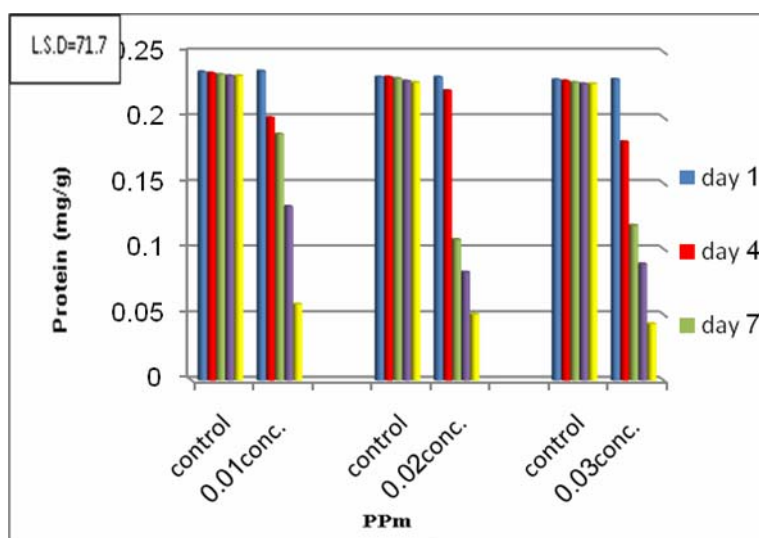


Figure 55. Variation in protein content of *P. perfoliatus* that exposed in different concentration of Reactive Blue dye mg/gm

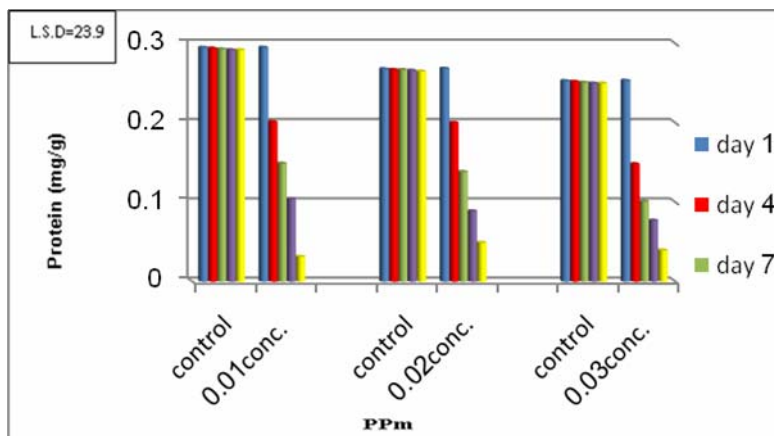


Figure 56. Variation in protein content of *P. perfoliatus* that exposed in different concentration of Reactive yellow dye mg/gm

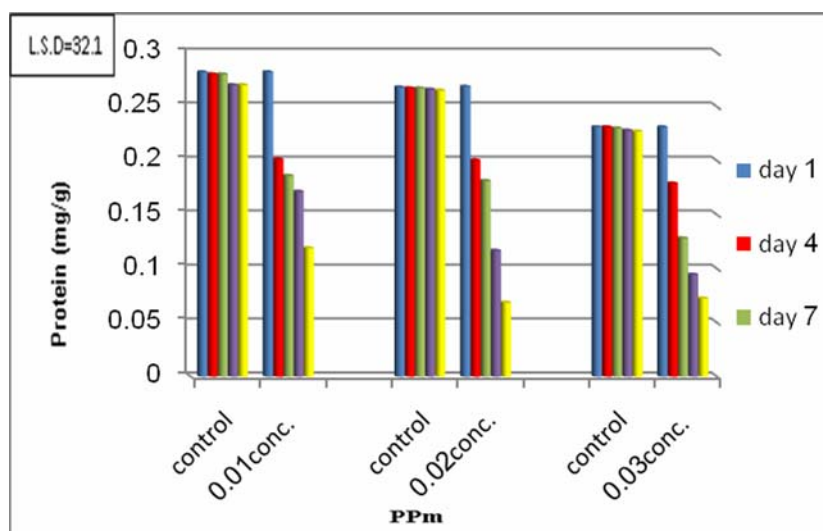


Figure 57. Variation in protein content of *P. perfoliatus* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes

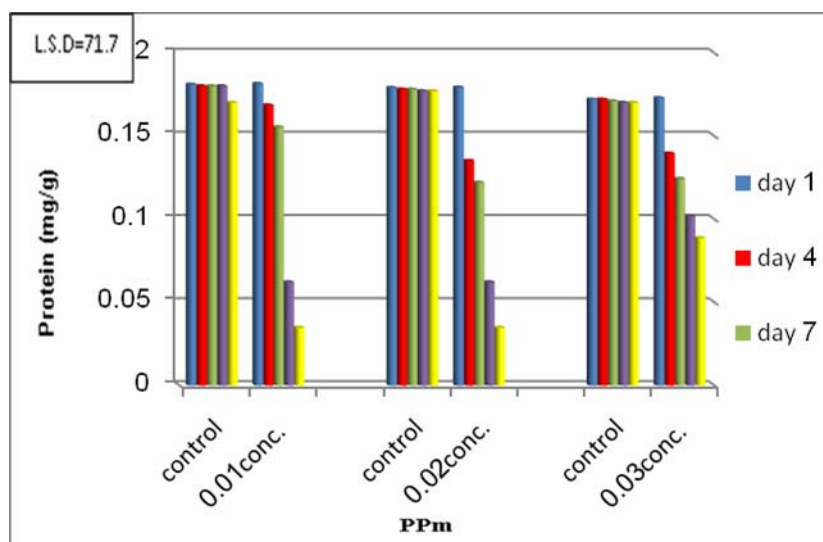


Figure 58. Variation in protein content of *H. varcillata* that exposed in different concentration of Reactive Blue dye mg/gm

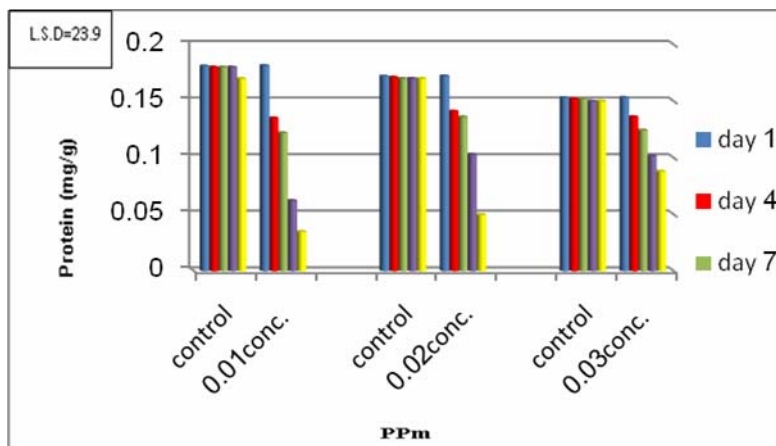


Figure 59. Variation in protein content of *H. varcillata* that exposed in different concentration of Reactive yellow dye mg/gm

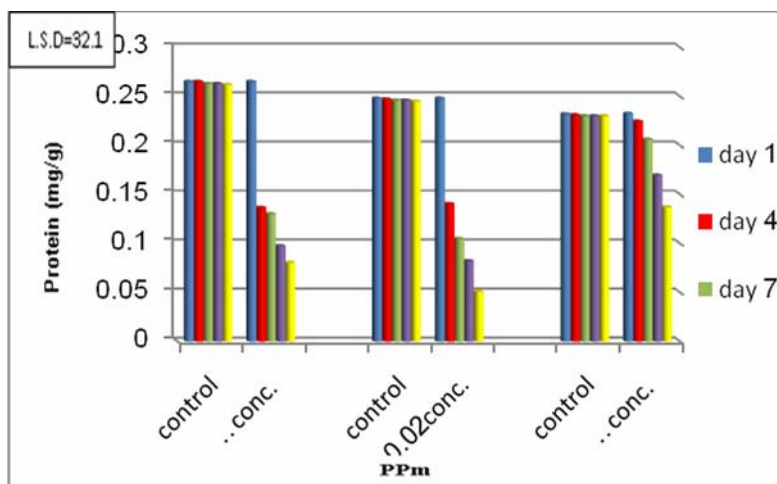


Figure 60. Variation in protein content of *H. varcillata* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes mg/gm

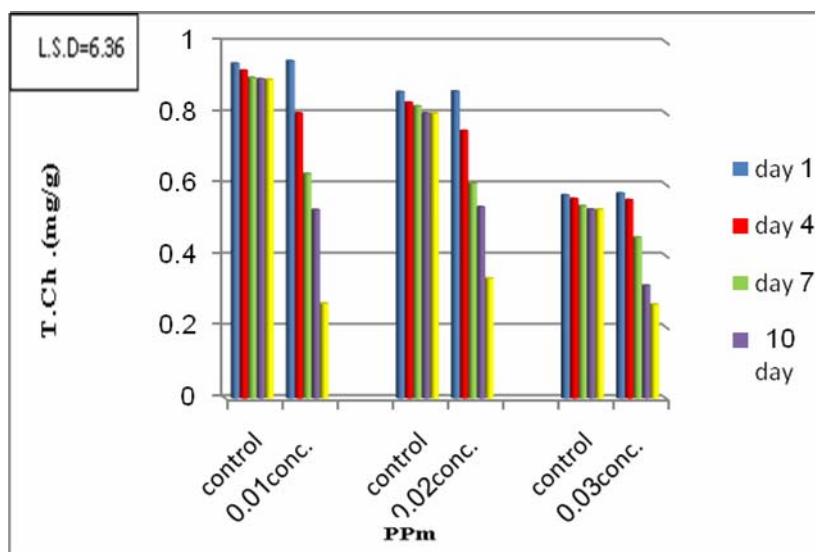


Figure 61. Variation in total chlorophyll of *P. australis* that exposed in different concentration of Reactive Blue dye mg/g .

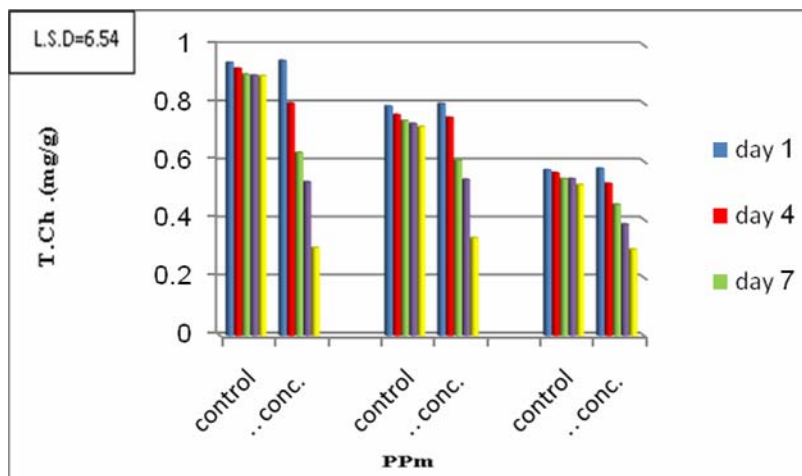


Figure 62. Variation in total chlorophyll of *P. australis* that exposed in different concentration of Reactive yellow dye mg/g .

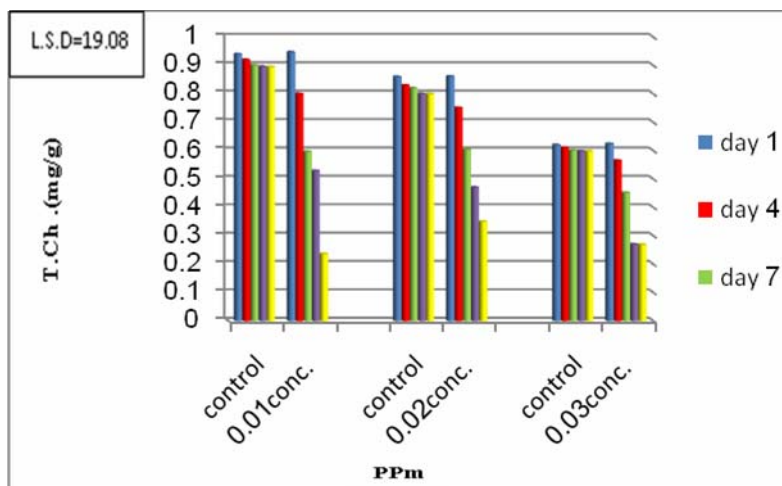


Figure 63. Variation in total chlorophyll of *P. australis* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dye

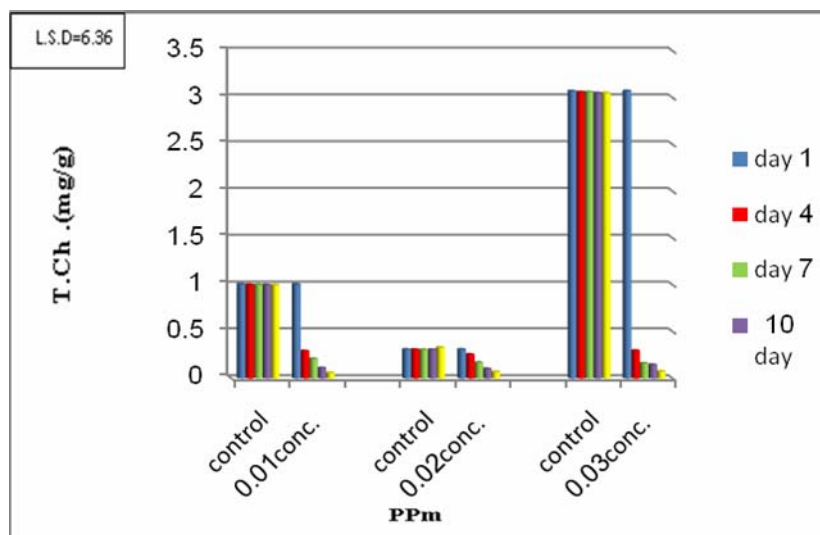


Figure 64. Variation in total chlorophyll of *T. domingensis* that exposed in different concentration of Reactive Blue dye mg/g .

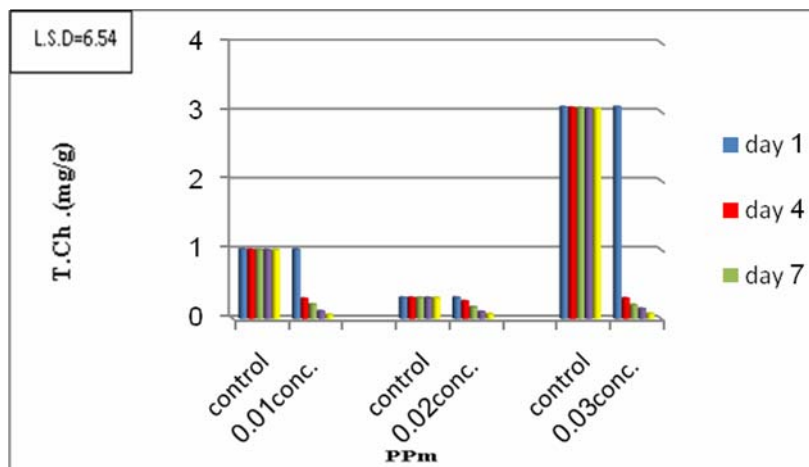


Figure 65. Variation in total chlorophyll of *T. domingensis* that exposed in different concentration of Reactive yellow dye mg/g .

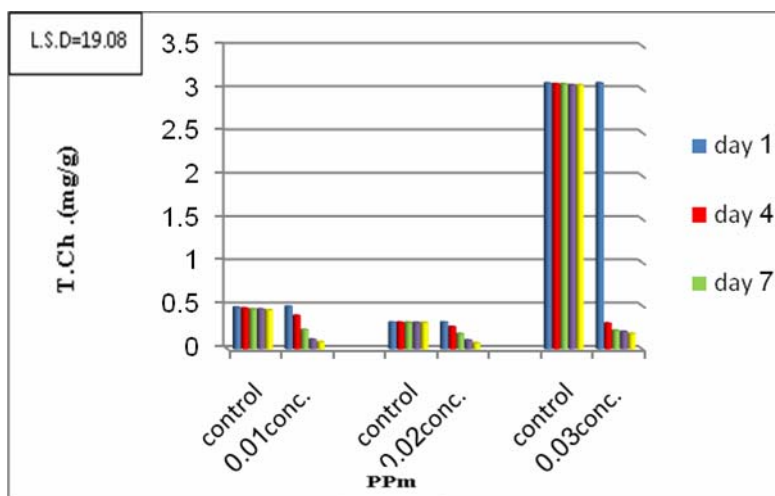


Figure 66. Variation in total chlorophyll of *T. domingensis* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dye mg/g .

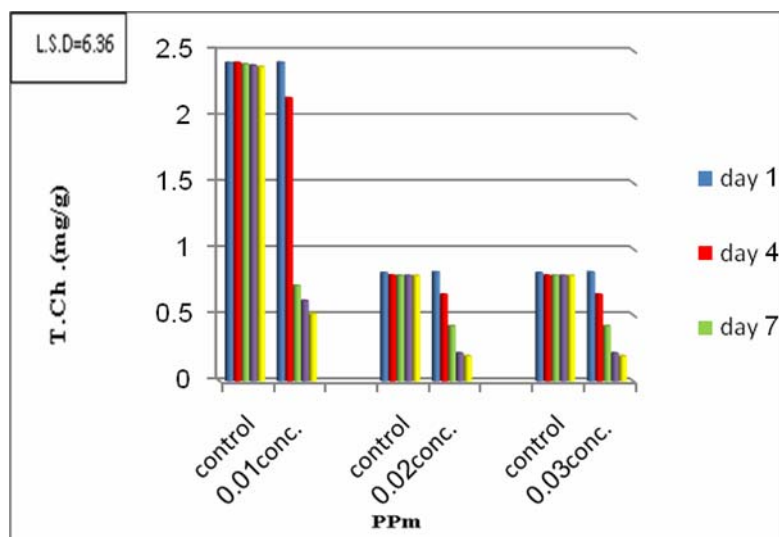


Figure 67. Variation in total chlorophyll of *C. demersum* that exposed in different concentration of Reactive Blue dye mg/g .

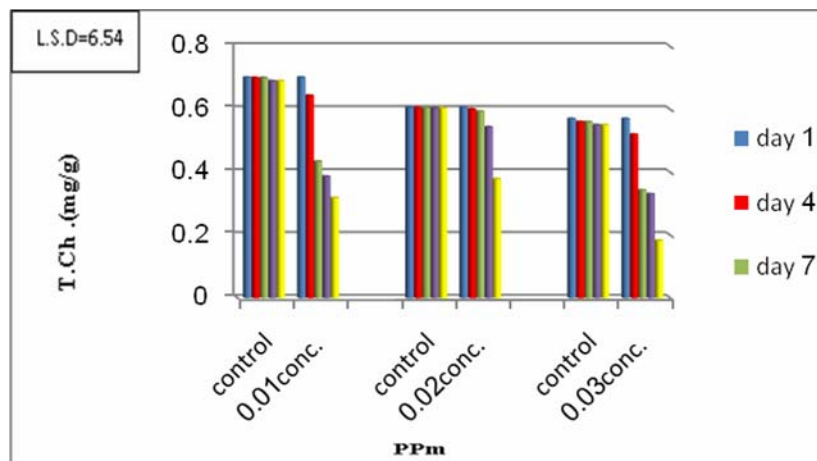


Figure 68. Variation in total chlorophyll of *C. demersum* that exposed in different concentration of Reactive yellow dye mg/g .

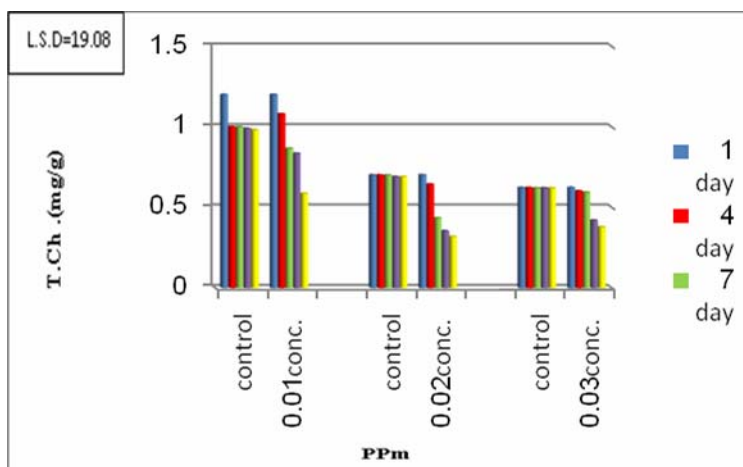


Figure 69. Variation in total chlorophyll of *C. demersum* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes mg/g .

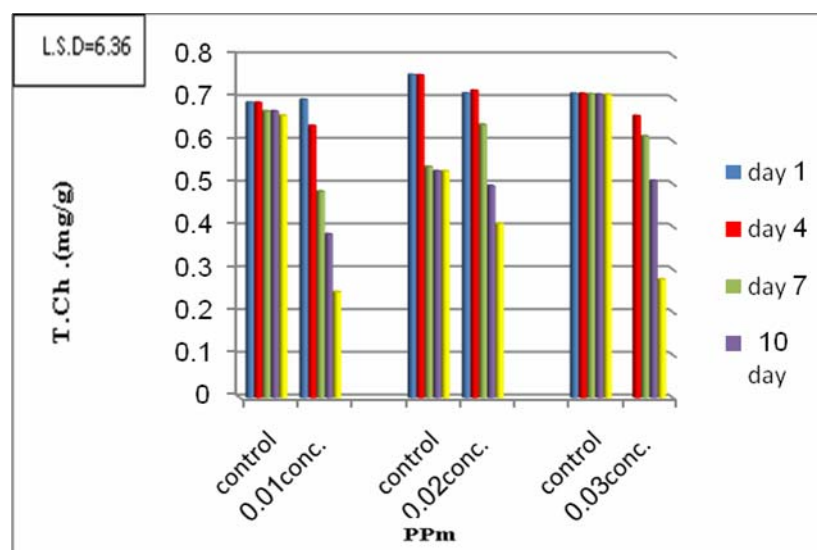


Figure 70. Variation in total chlorophyll of *P. perfoliatus* that exposed in different concentration of Reactive Blue dye mg/g .

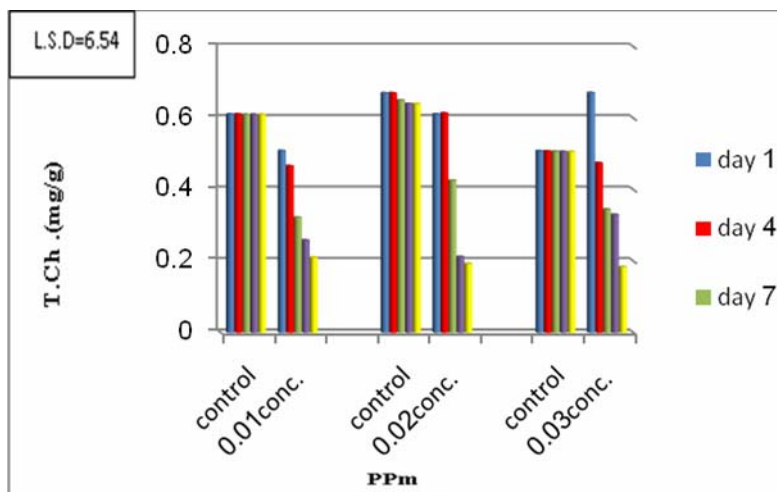


Figure 71. Variation in total chlorophyll of *P. perfoliatus* that exposed in different concentration of Reactive yellow dye mg/g .

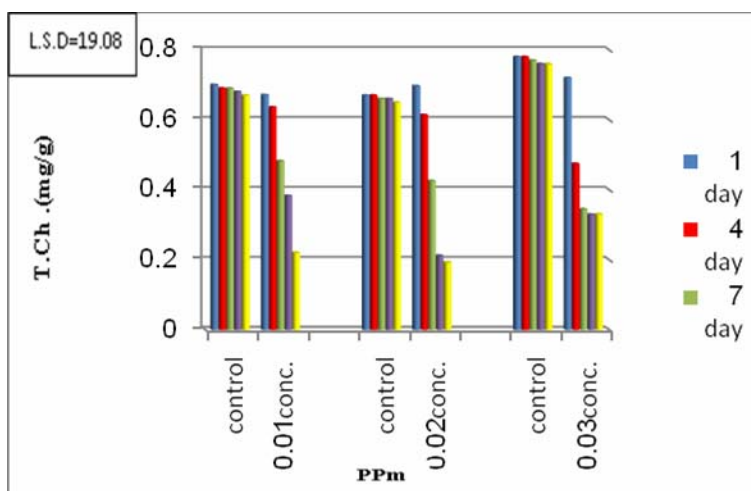


Figure 72. Variation in total chlorophyll of *P. perfoliatus* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes mg/g .

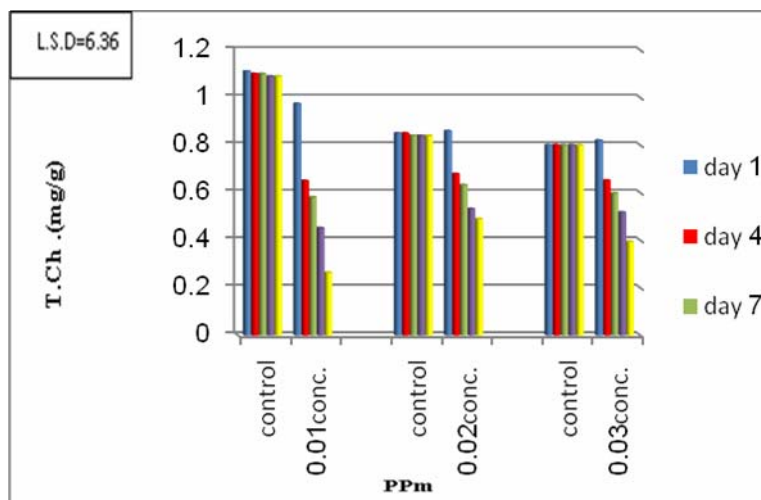


Figure 73. Variation in total chlorophyll of *H. varcillata* that exposed in different concentration of Reactive Blue dye mg/g

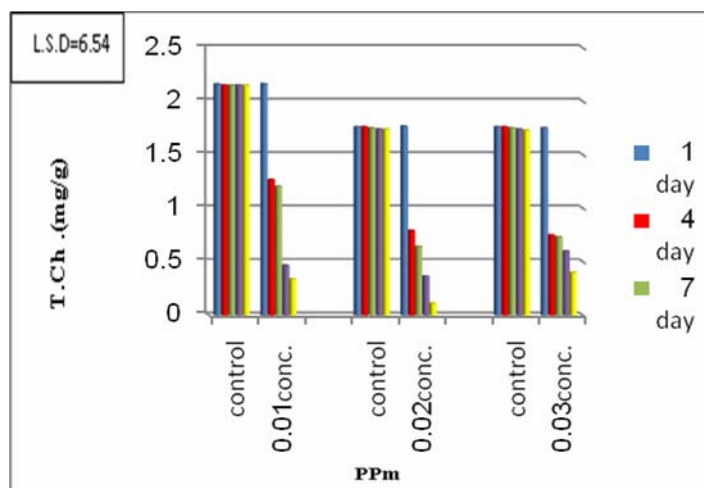


Figure 74. Variation in total chlorophyll of *H. varcellata* that exposed in different concentration of Reactive yellow dye mg/g

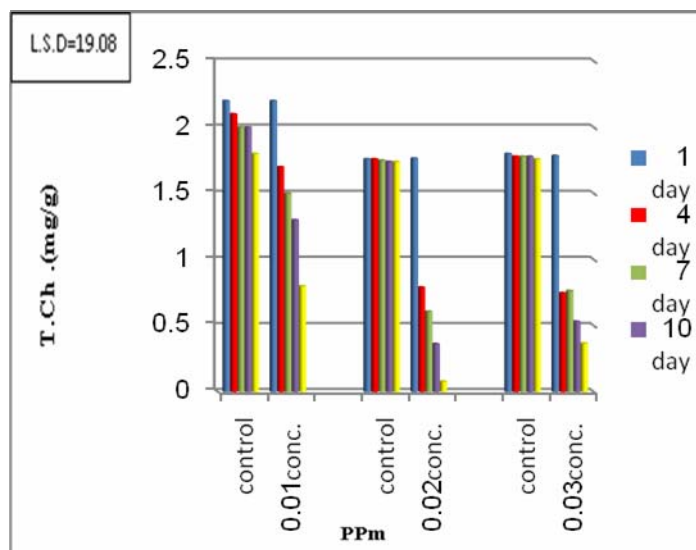


Figure 75. Variation in total chlorophyll of *H. varcellata* that exposed in different concentration of mixture (Reactive Blue, Reactive yellow) dyes mg/g .

Conclusions

Aquatic plants developed a complex antioxidant defense system against abiotic stress for instance contaminants . the study show decrease in total chlorophyll and protein content in aquatic plants and high content in malondialdehyde and increasing the activity of catalase and superoxide dismutase enzyme in aquatic plants .

Acknowledgement

The authors are gratefull for department of Biology in collage of science Babylon university.

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